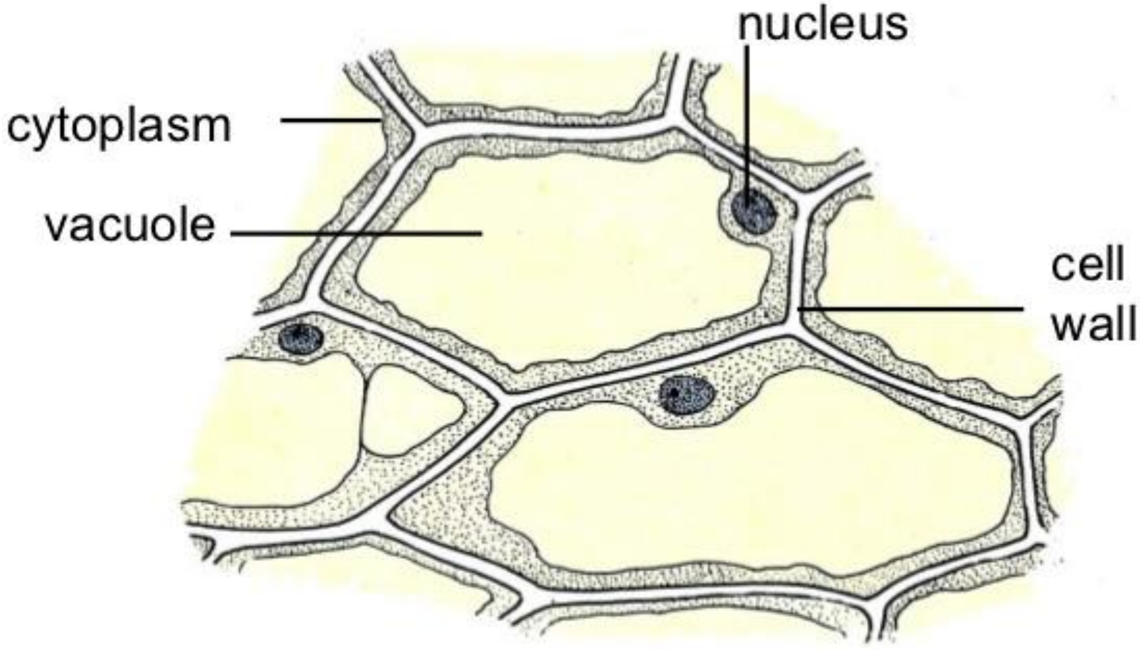
4th Lesson, Pathology

The fungal mechanism contributing to fungal host colonization

Activation of fungal pathogenicity factors during ripening fruits

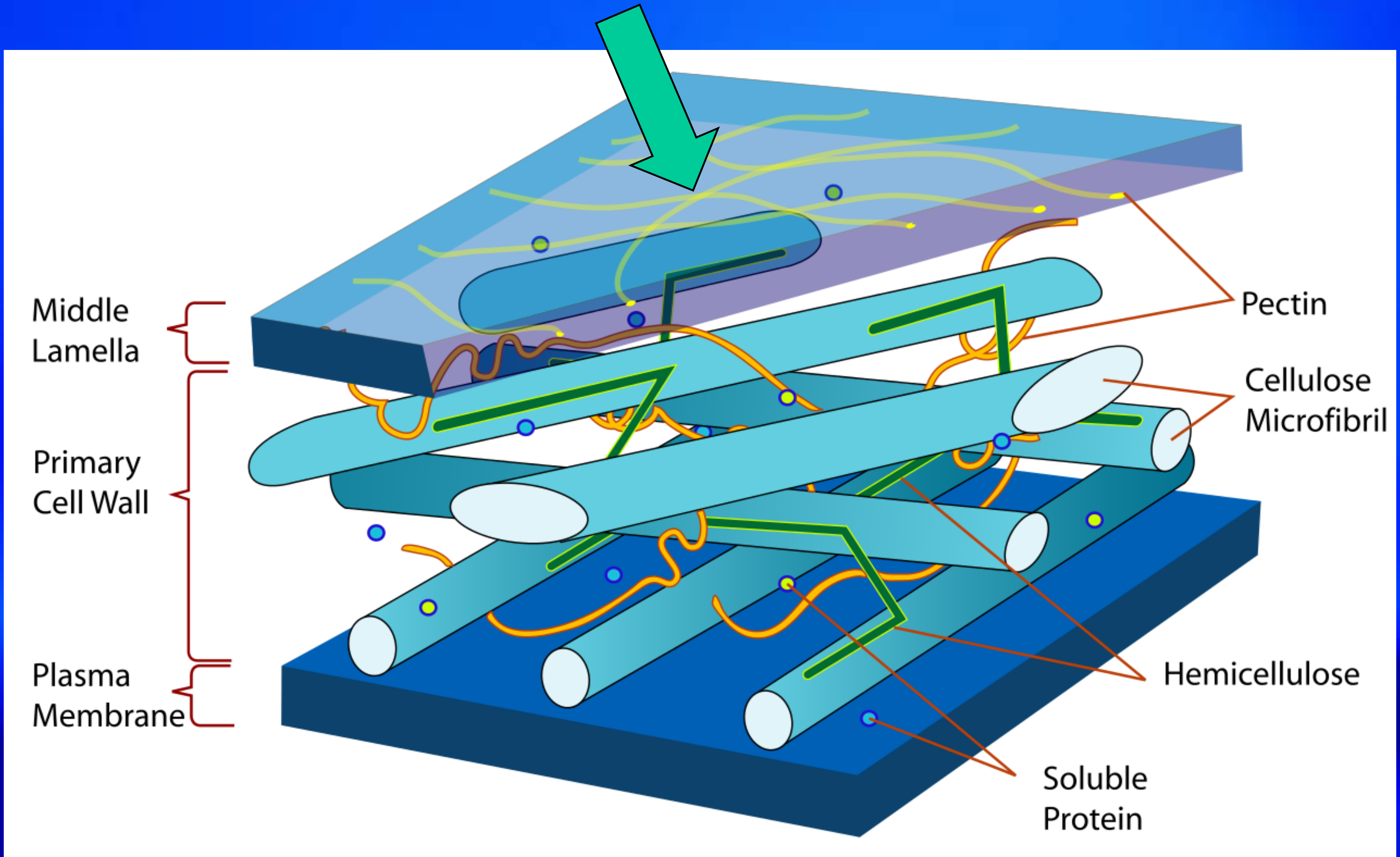
Fruit decay include fruit maceration

Plant cells

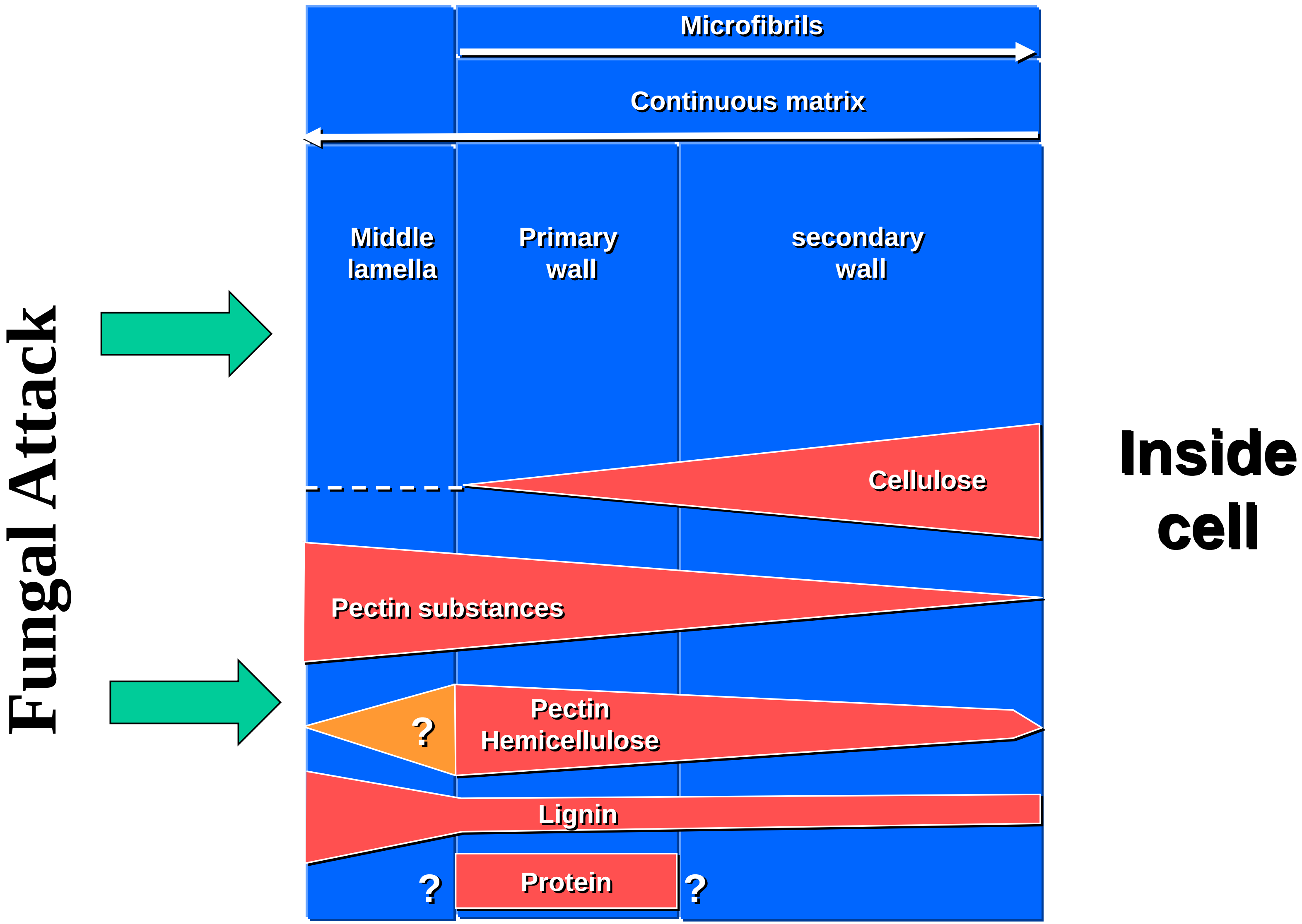


Plant cells differ from animal cells in having a cell wall outside the cell membrane, and a large, fluid-filled vacuole

Cell wall of the plant



General Cell wall structure



Pectic materials in the cell walls

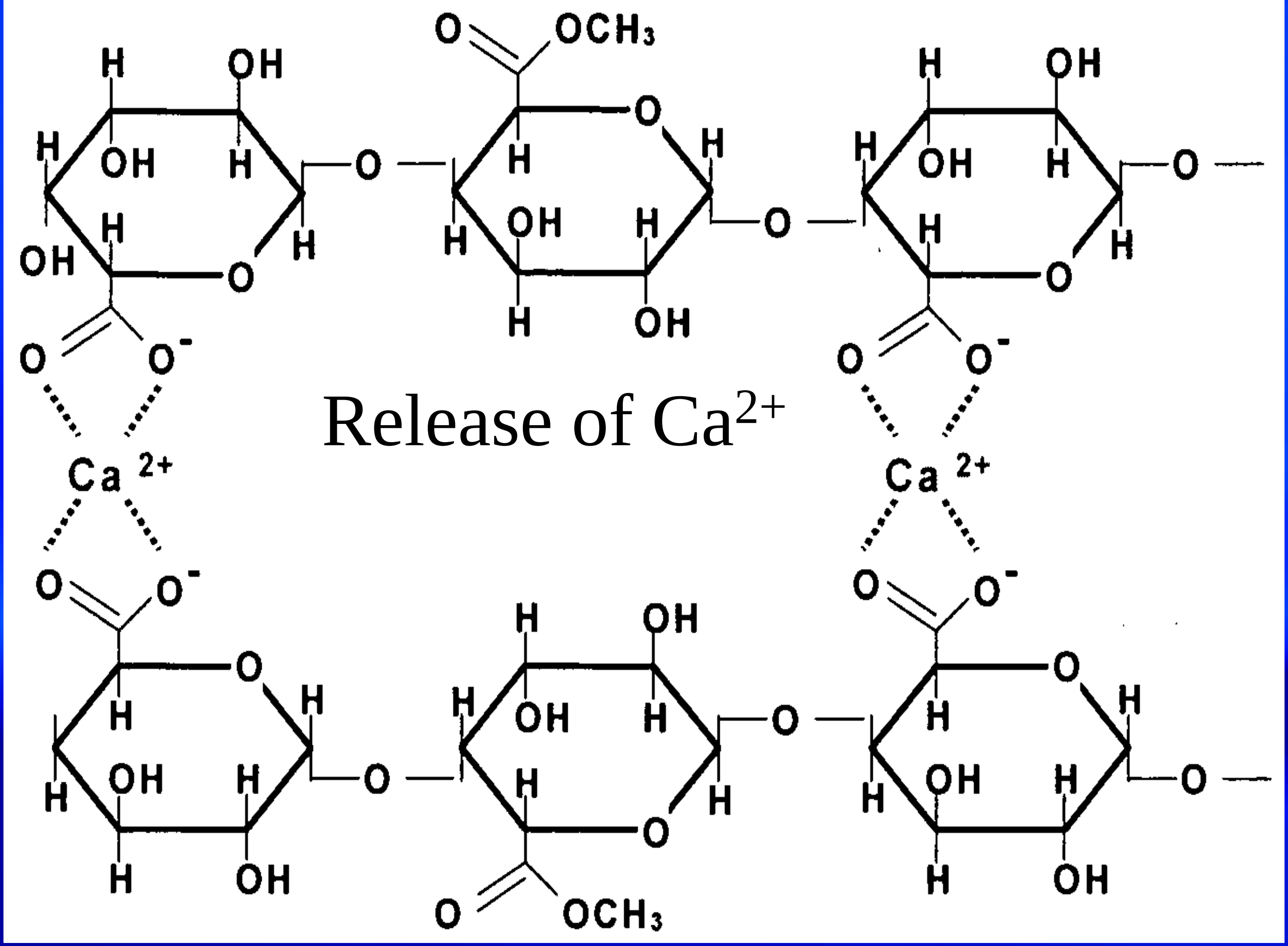
- **Pectic material** is the main component of the middle lamella and primary wall.
- Pectic material contains α-1,4- galacturonic acid chains with rhamnose α-1,2 linkage
- 16% rammnogalacturonam
- 9% galactan, galactose in β-1,4 linkage
- 9% arabinan, arabinose in α-1,5 linkage

- **Cellulose and hemi-cellulose** is the main component of the secondary wall
- Glucose in β-1,4 linkage

Pectolytic enzymes

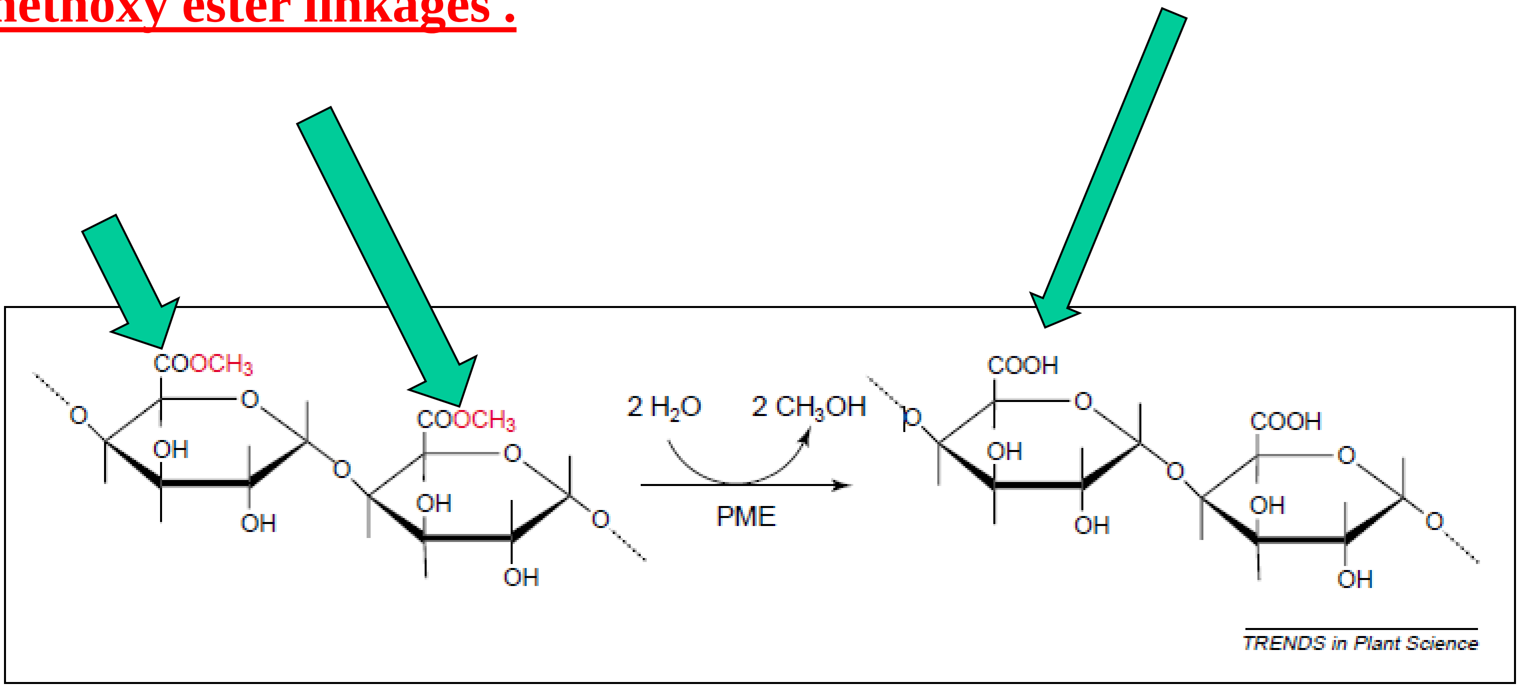
- Pectin methyl esterases (PME)
- Polyglacturonase (PG)
- Pectate lyase (PL)

PME activity first step: The release of Ca^{2+} between the glacturonic acid chains



Pectin Methylesterases second step:

Pectin methylesterase catalyzes the de-esterification through transferring the C6 carboxyl groups to water molecules altering the degree and pattern of methyl esterification and resulting in the formation of high molecular weight pectins with new **non-methoxy ester linkages .**

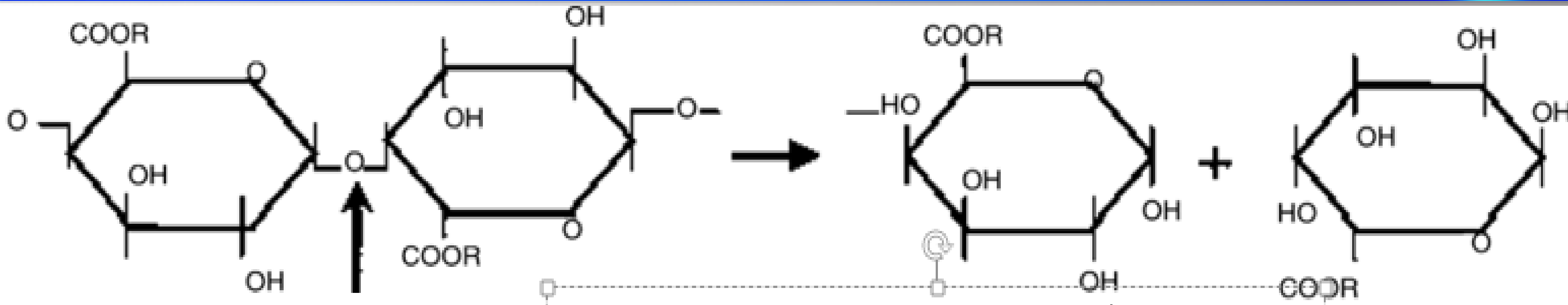


Pectolytic enzymes

Polyglacturonase (PG)
Pectate lyase (PL)

Both catalyze the reaction at the α-1,4 binding site

PG by hydrolysis
PL by trans-elimination

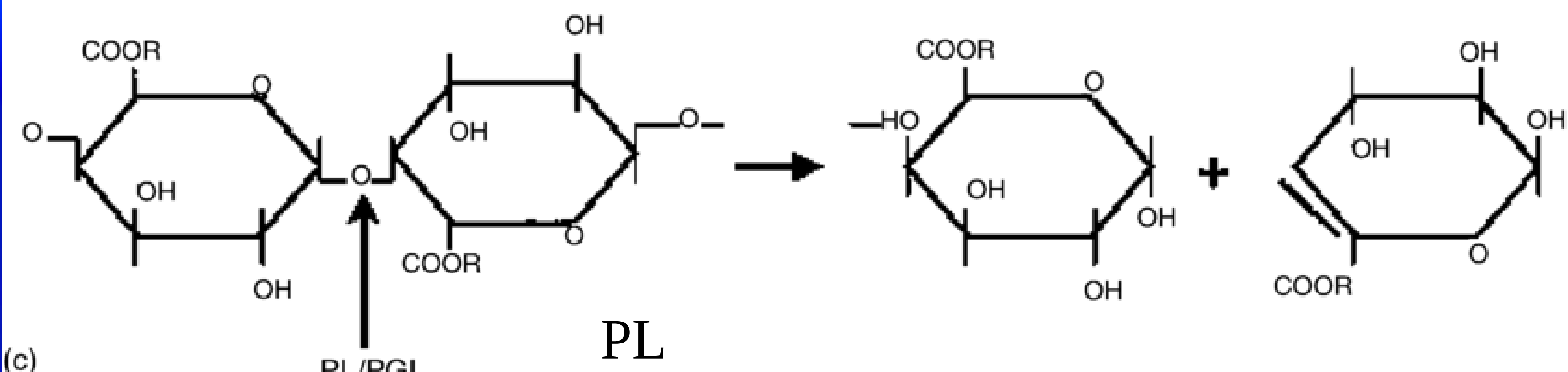


(a)

PG

Simple hydrolysis

Hydrolysis by trans-elimination



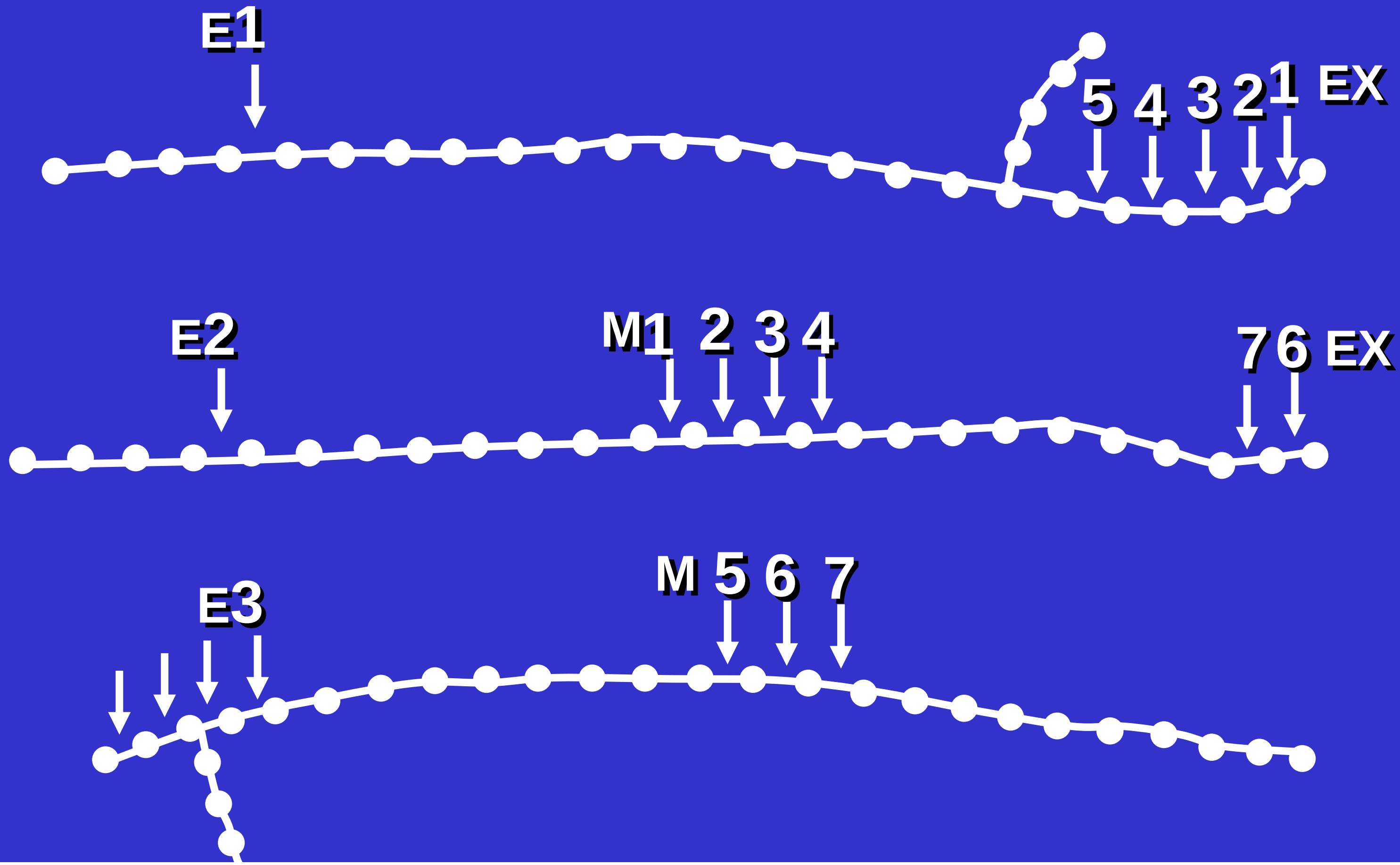
(c)

PL

Pectolytic enzymes

- Pectolytic activity with endo and exo activity

Endo and exo digesting enzymes



Inducible enzymes

- **Pectolytic enzymes and inducible enzymes**

In vivo and Vitro

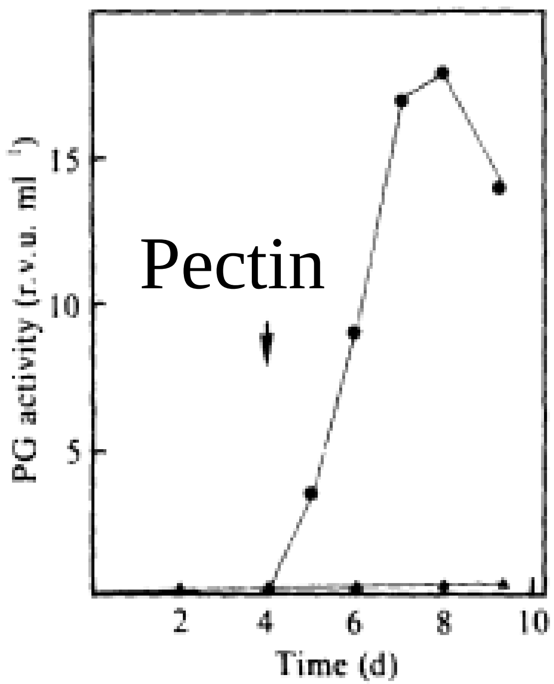
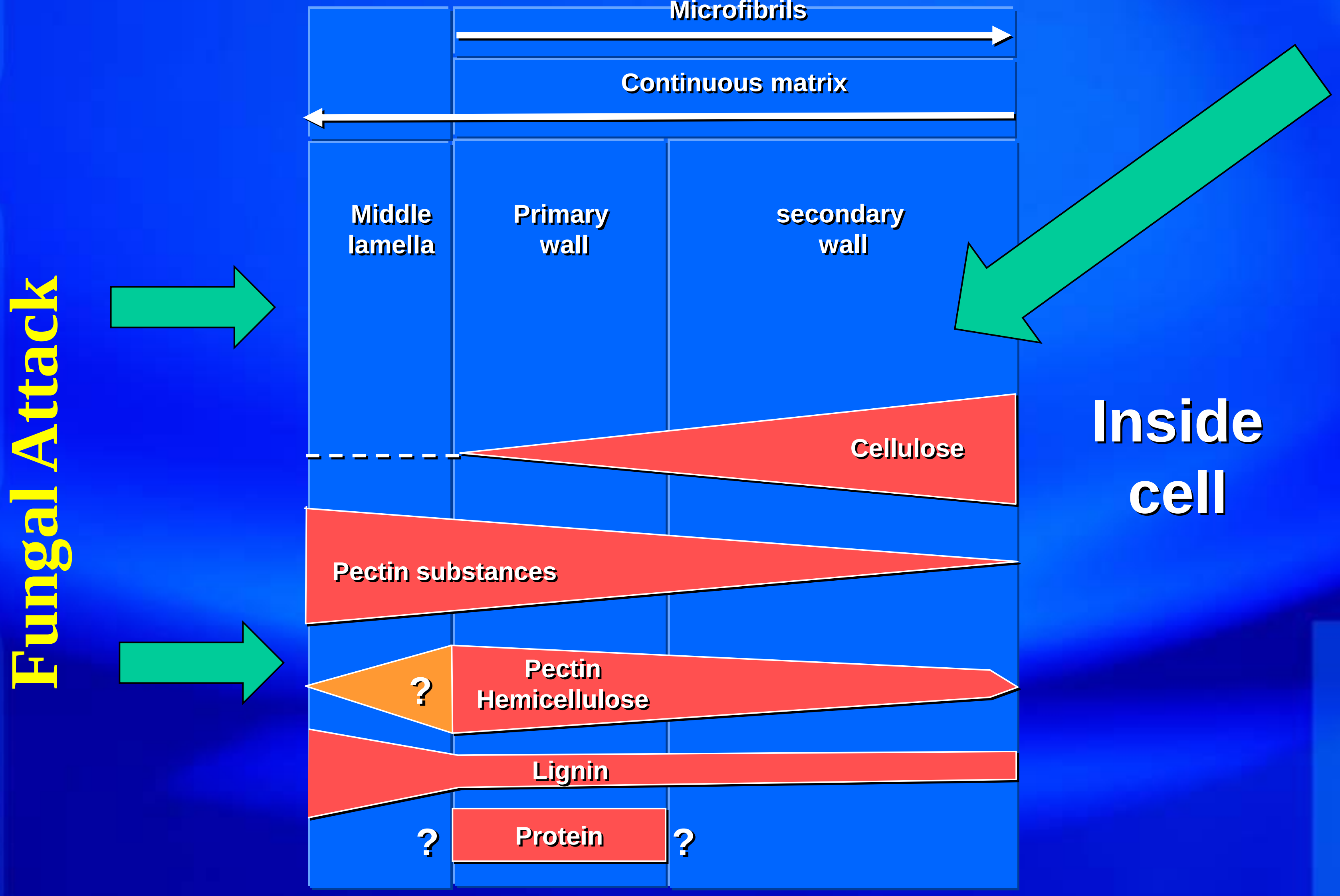
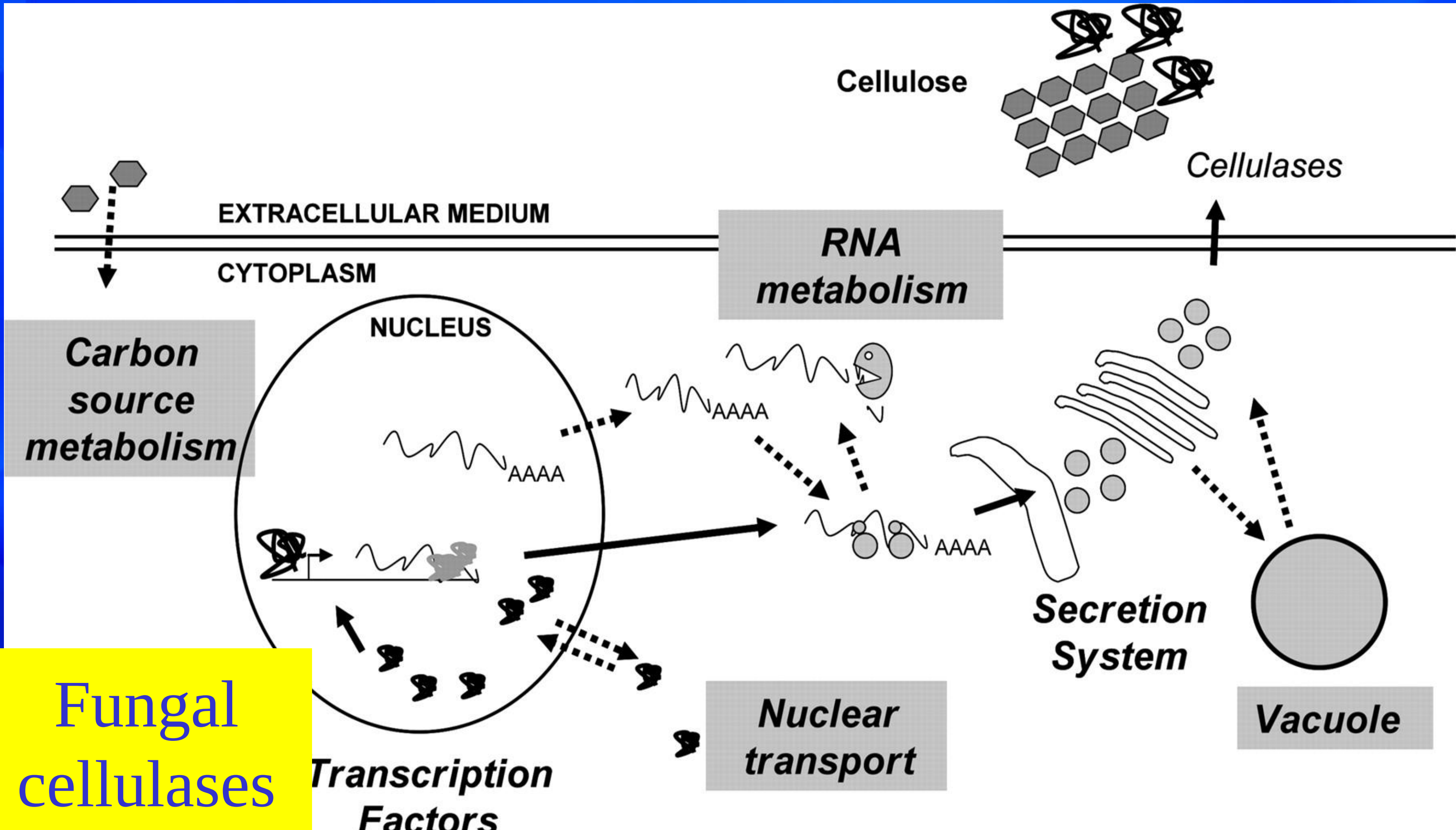


Fig. 1. Time-course of PG induction by pectin in glucose-grown *F. moniliforme*. After 4 d of growth in glucose, the mycelium was transferred to a pectin-containing medium. The mycelium was harvested 2 d later and mRNA was isolated. Other experimental conditions are described in the text. ●, Pectin-induced; ▲, non-induced.

General Cell wall structure

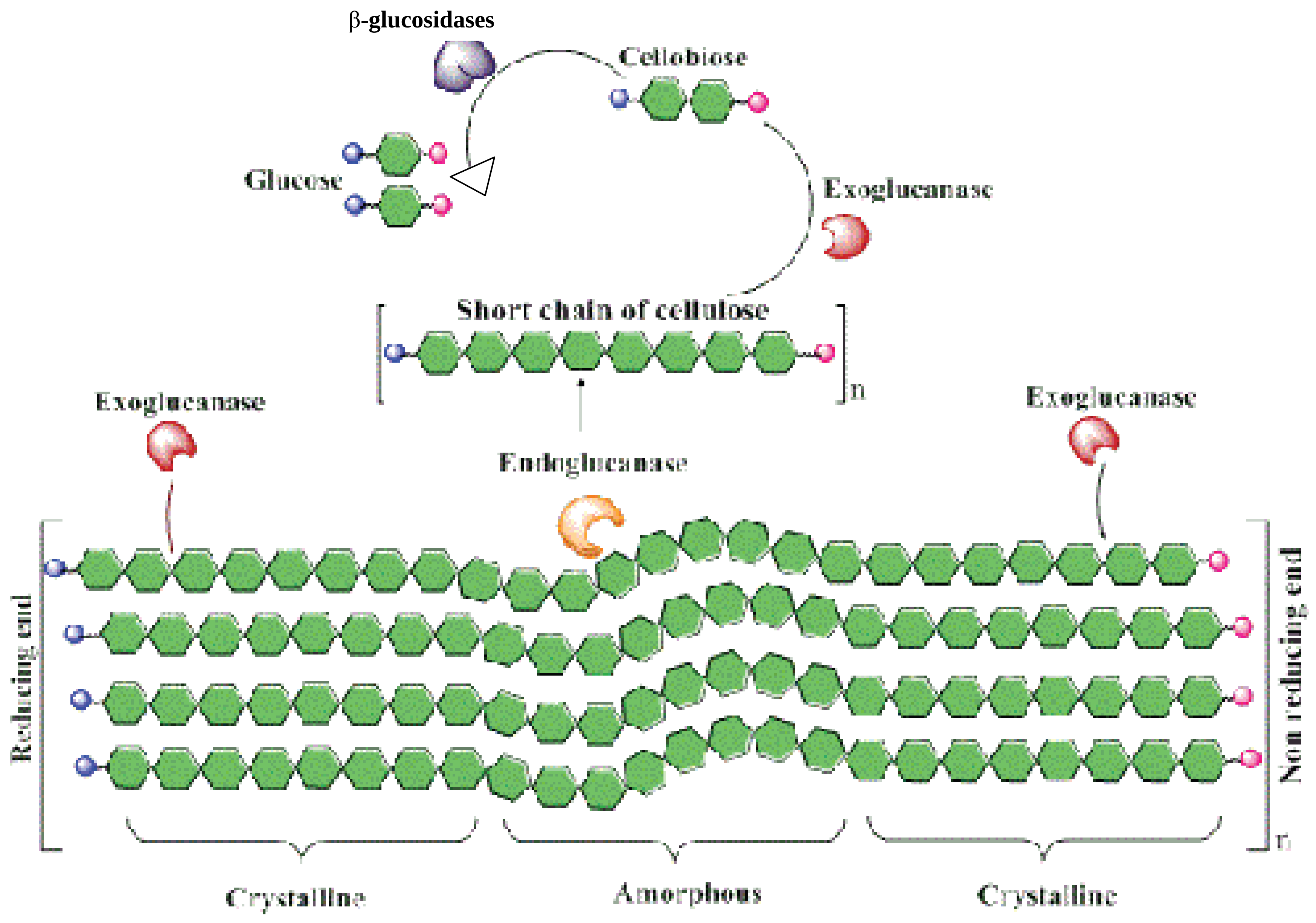


Penetration of the Host secondary wall



Fungal cellulases

Fungal Cellulase



Pathogenicity of Postharvest pathogens

Pathogenicity of postharvest pathogens: Methods of study

- Gene expression
- Development of mutants with inhibited secretion
- Development of mutant with disrupted genes

Pectolytic enzymes as enzymes contributing to pathogenicity: gene expression

Penicillium sp.
Sclerotinia sclerotiorum
Colletotrichum gloeosporioides

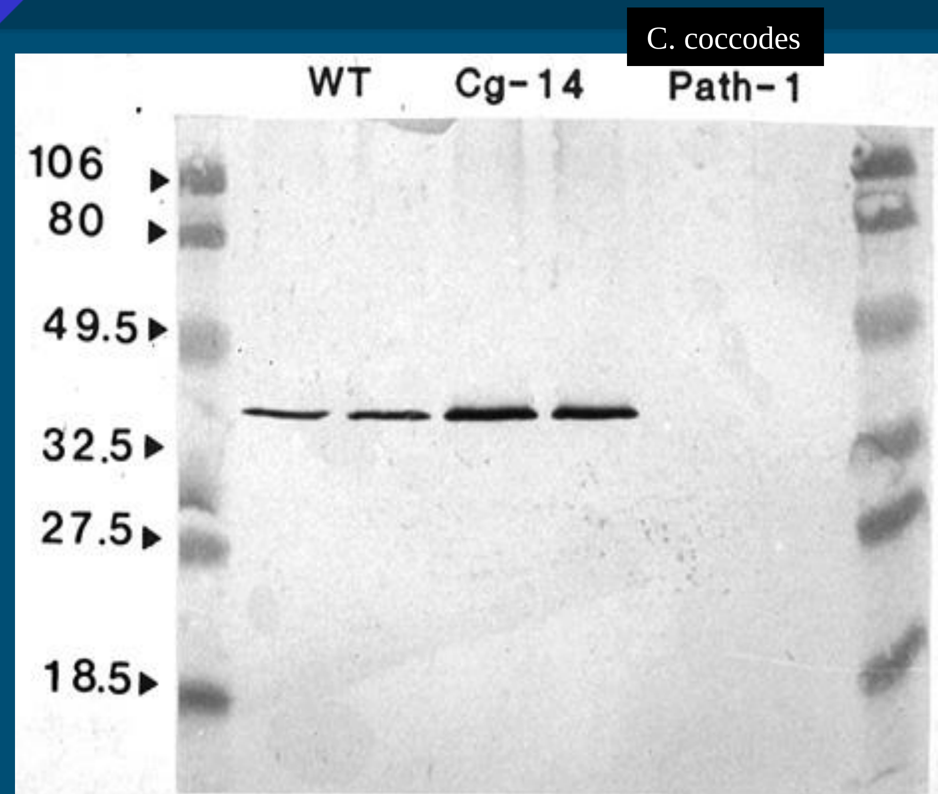
Development of an non-secreting mutant of Colletotrichum

Cg -14

WT

Path - 1

C. coccodes

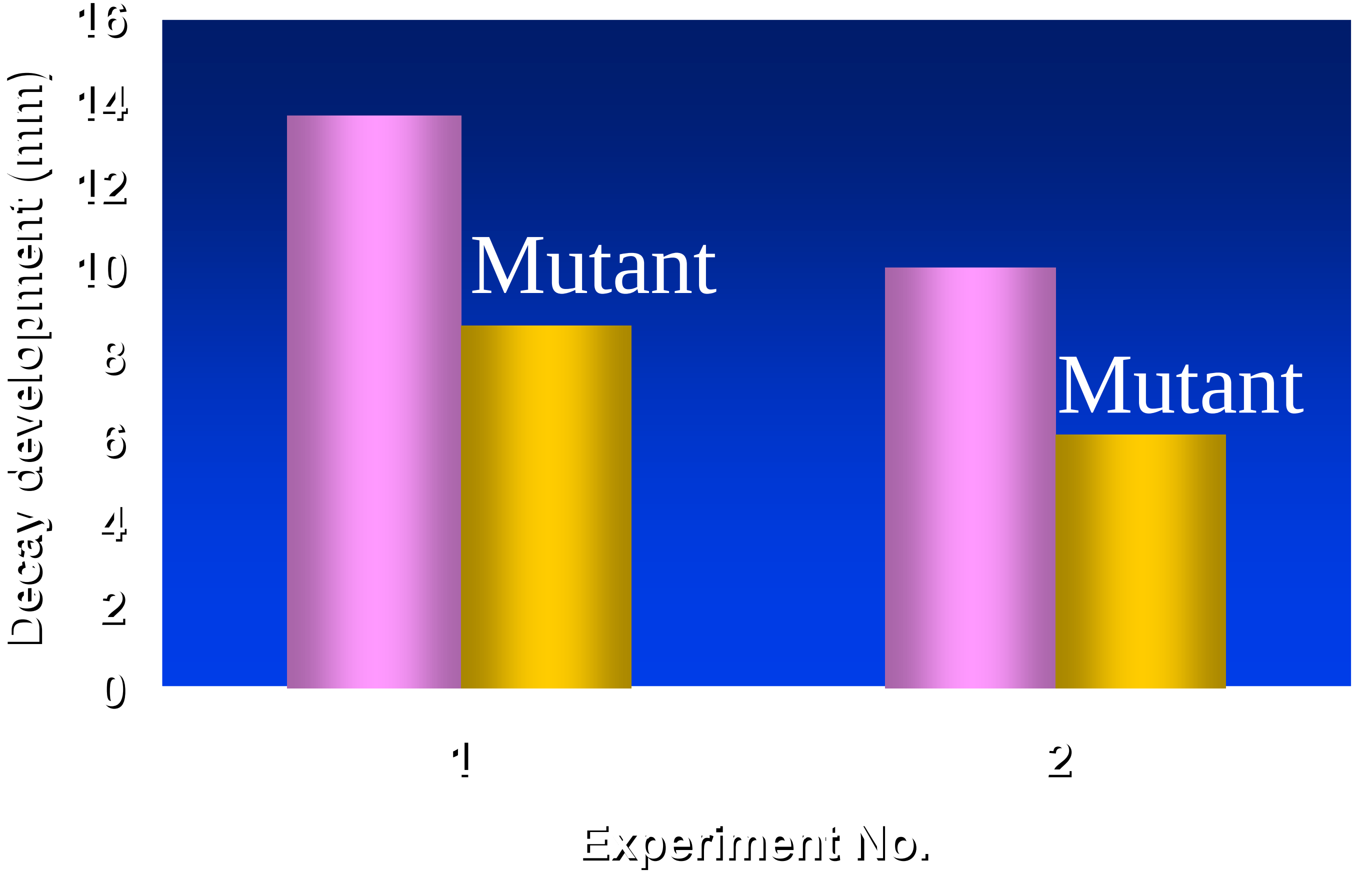


Pathogenicity of postharvest pathogens: Methods of study

- Gene expression
- Development of mutants with inhibited secretion
- Development of mutant with disrupted genes

Western blot of Pectate Lyase secreted by *C. gloeosporioides*, the disrupted mutants and the Hyg-resistant non disrupted mutant

Decay development affected by mutants
*C. gloeosporioides pel*B mutant Cg-14



Pathogenicity factors expressed in postharvest pathogens

PL is a gene that contribute to 30-40% of infection capabilities of *Colletotrichum*

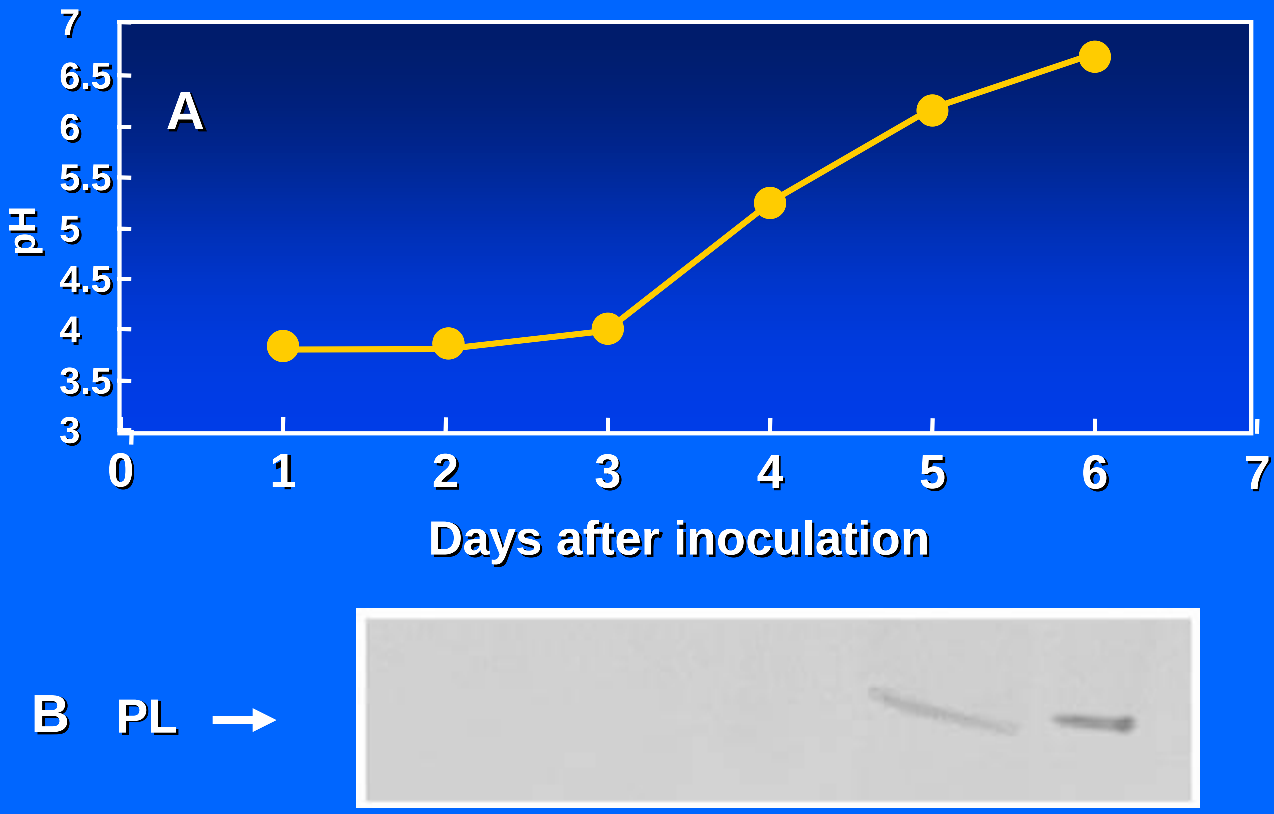
Other enzymes contribute to the other part ot the maceration capabilities of the fungus

Regulation of Pathogenicity factors by postharvest pathogens

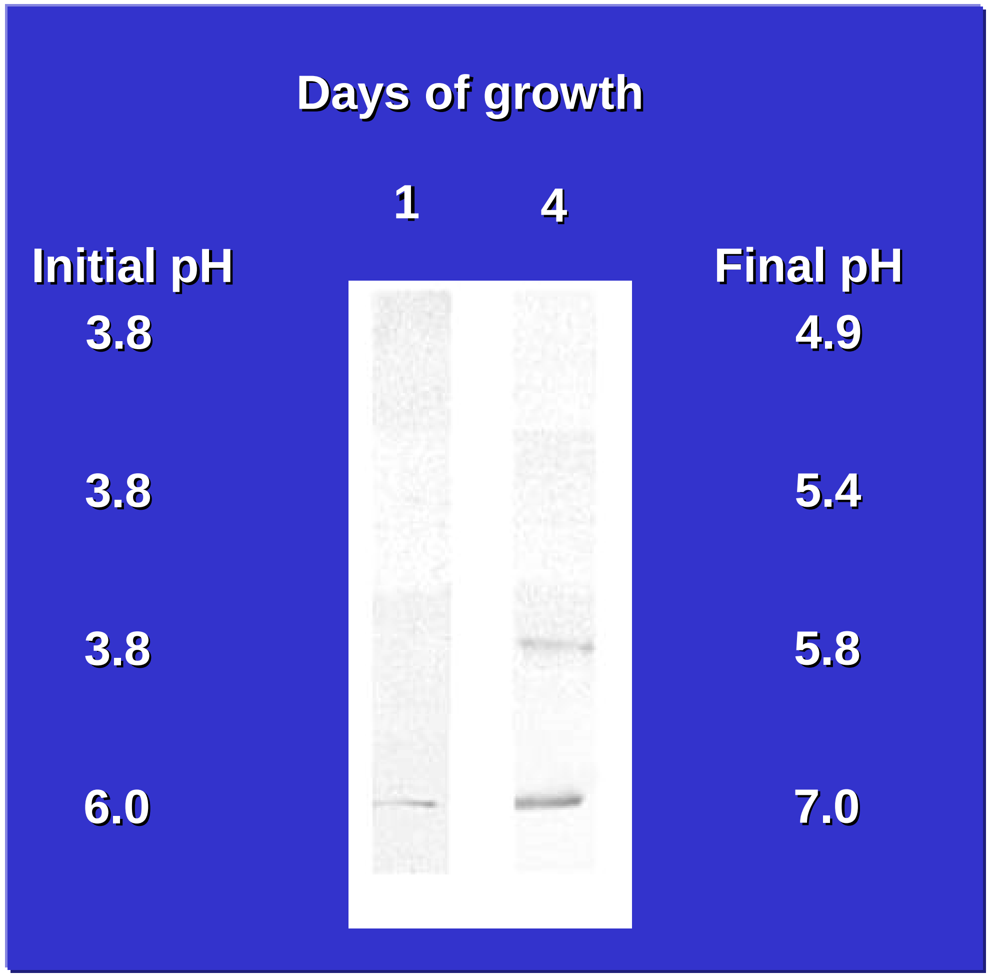
The regulation of pH

Changes in media pH during *C. gloeosporioides* growth

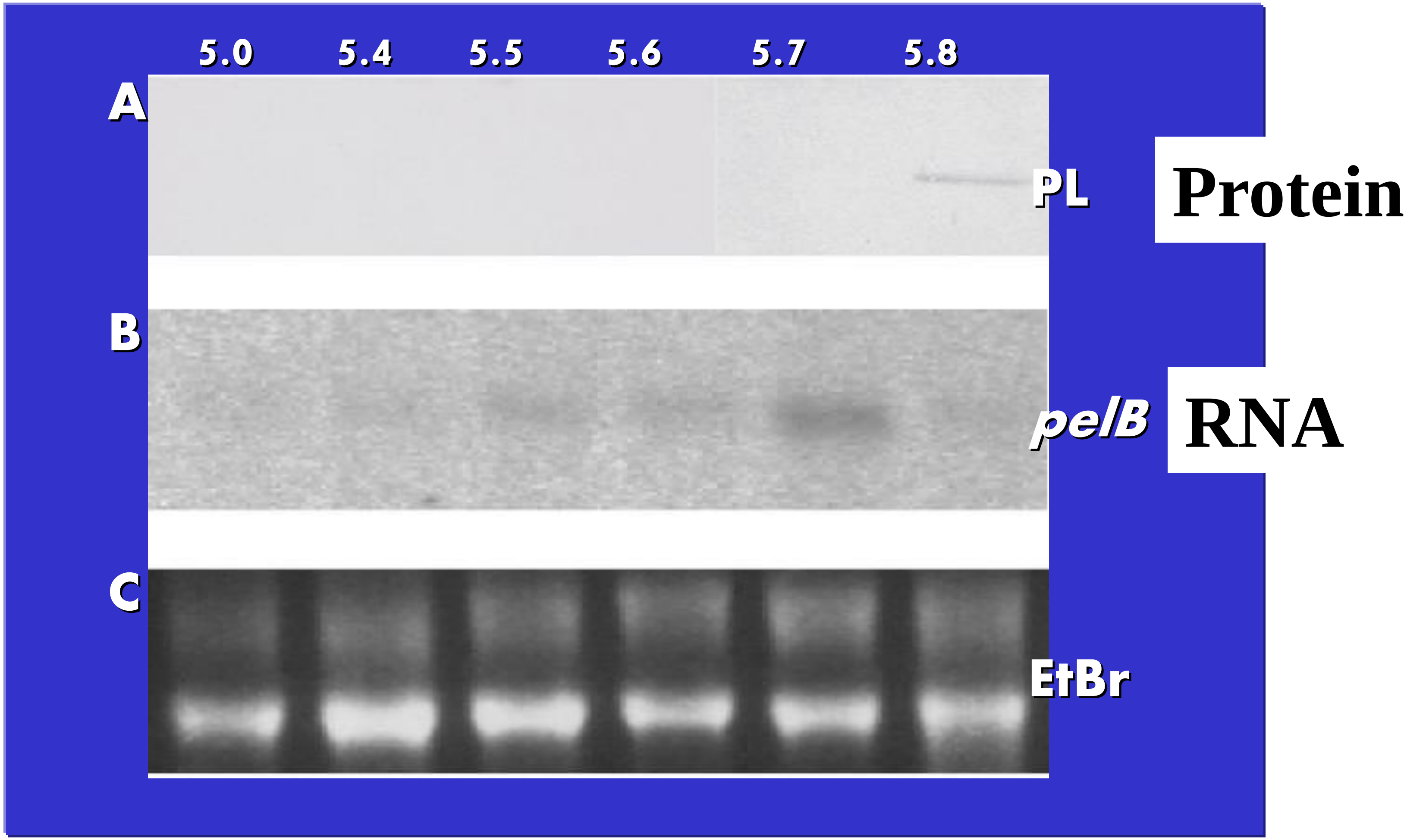
Acidic yeast extract media at pH 4.0



Effect of media pH on Pectate Lyase secretion by *C. gloeosporioides*

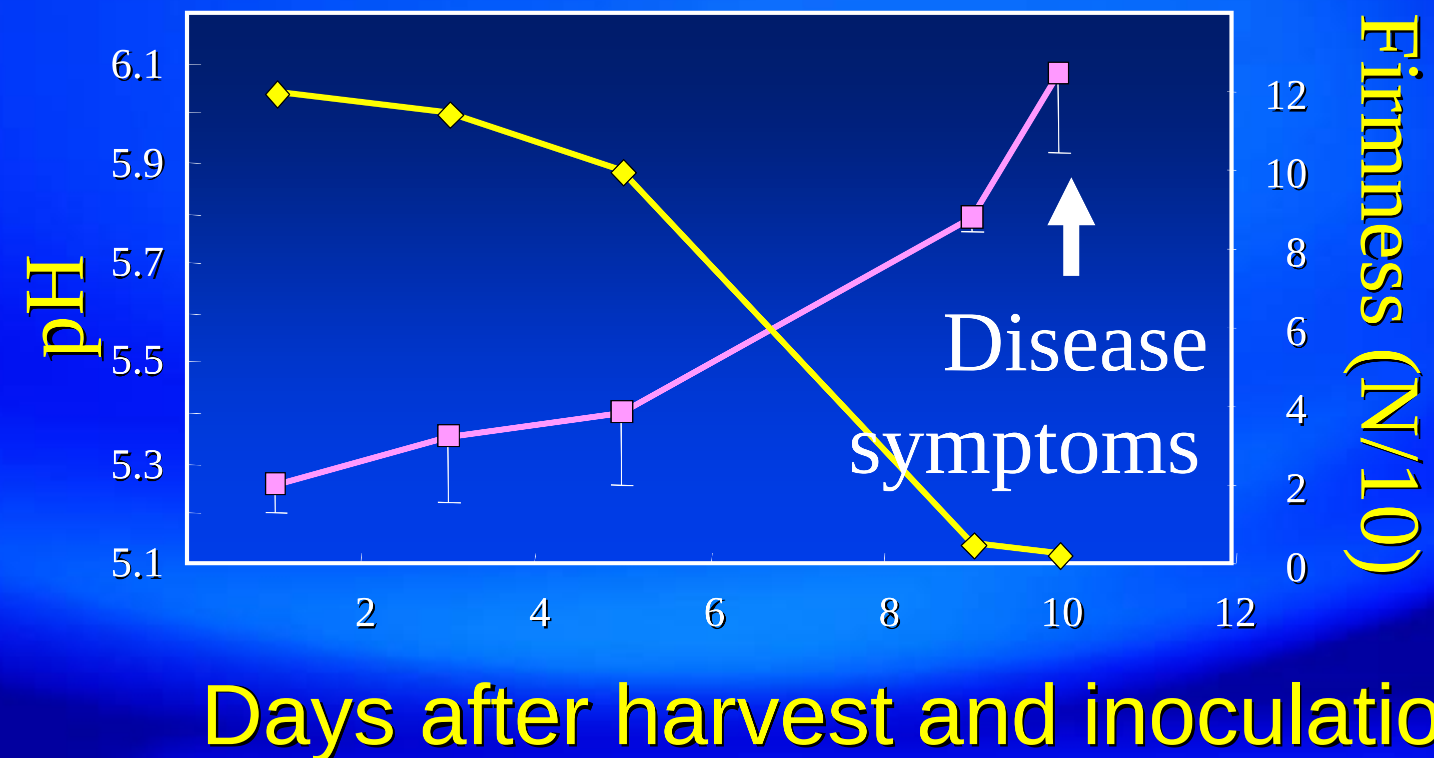


Expression of pelB and secretion of PL by *C. gloeosporioides* as a function of pH



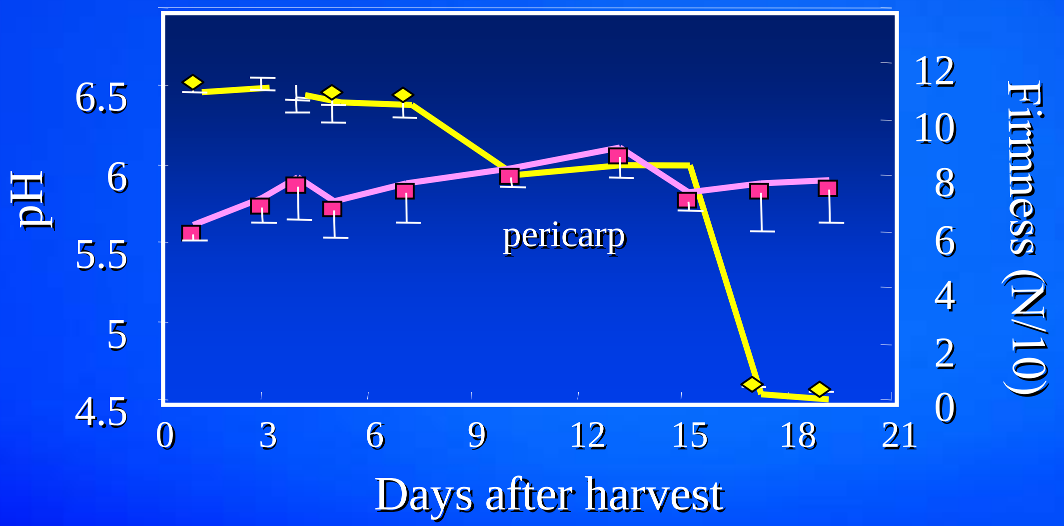
Is the host capable of modulating the differential expression of pectolytic enzymes so the fungus modulate it’s pathogenicity

Changes in pH at the peel of the avocado fruit cv. Fuerte during fruit ripening

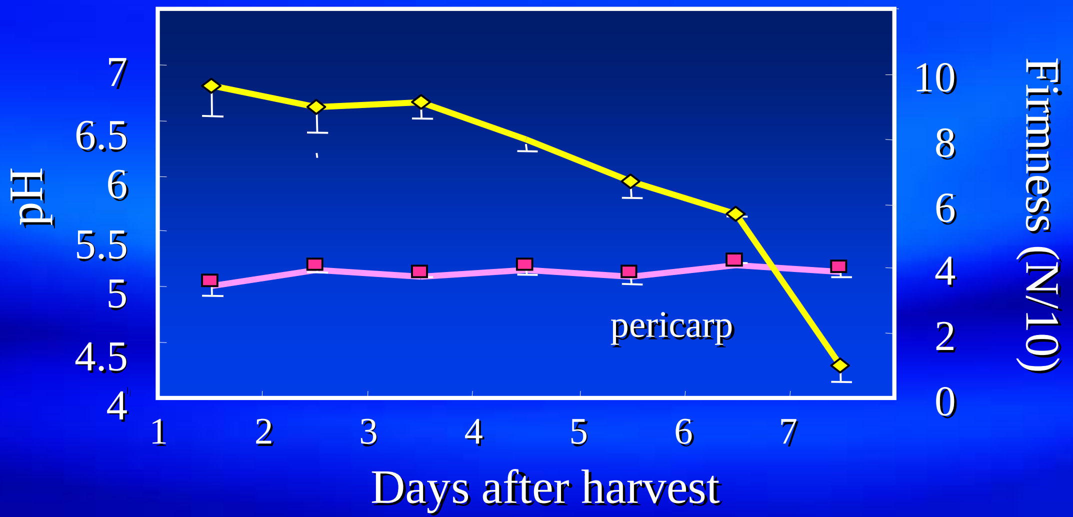


Changes in pH of two cultivars of avocado fruits

Fuerte

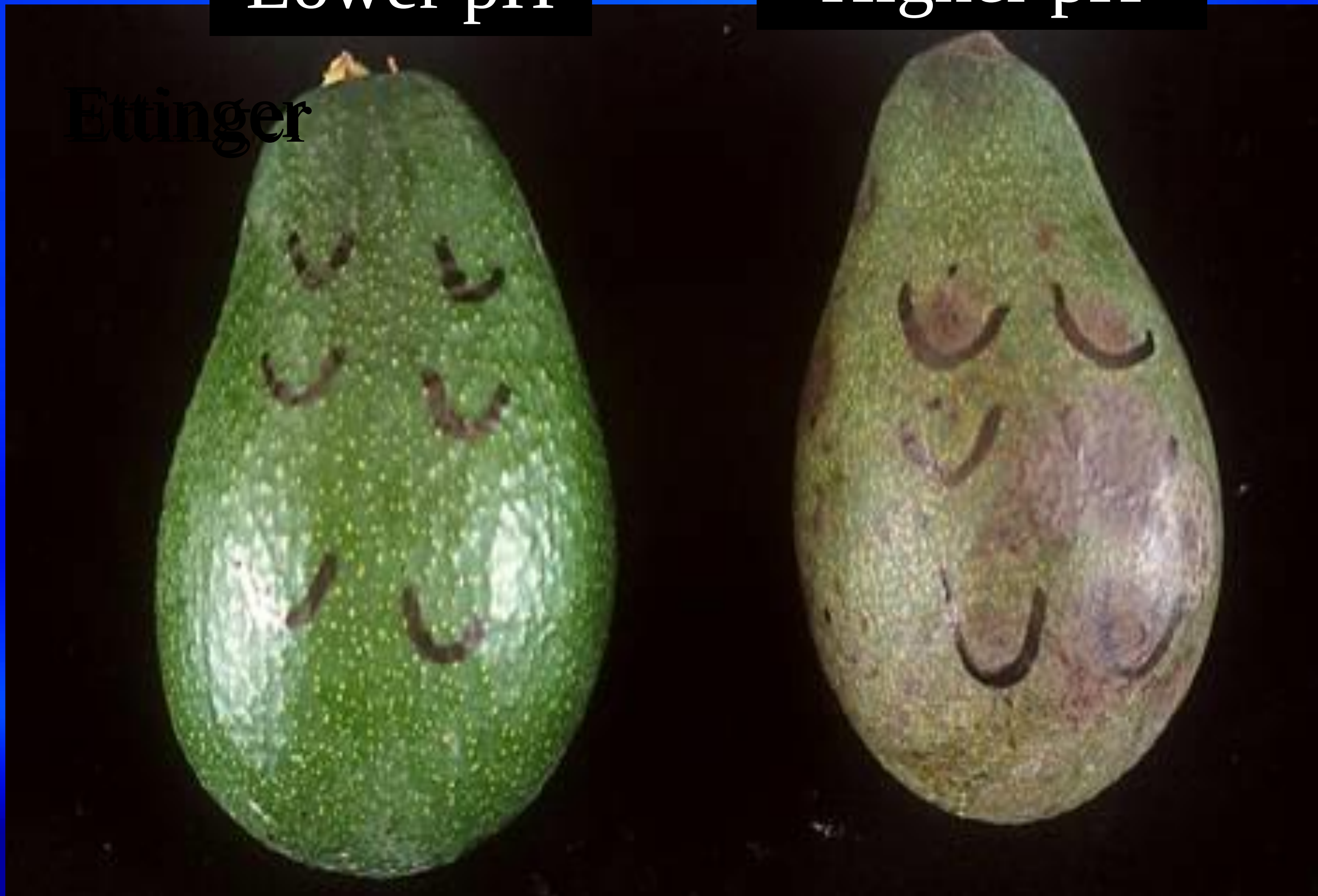


Ettinger



Ettinger
Lower pH

Fuerte
Higher pH



cv. Ettinger

Pectate lyase
inducing media at pH 3.8

cv. Ettinger

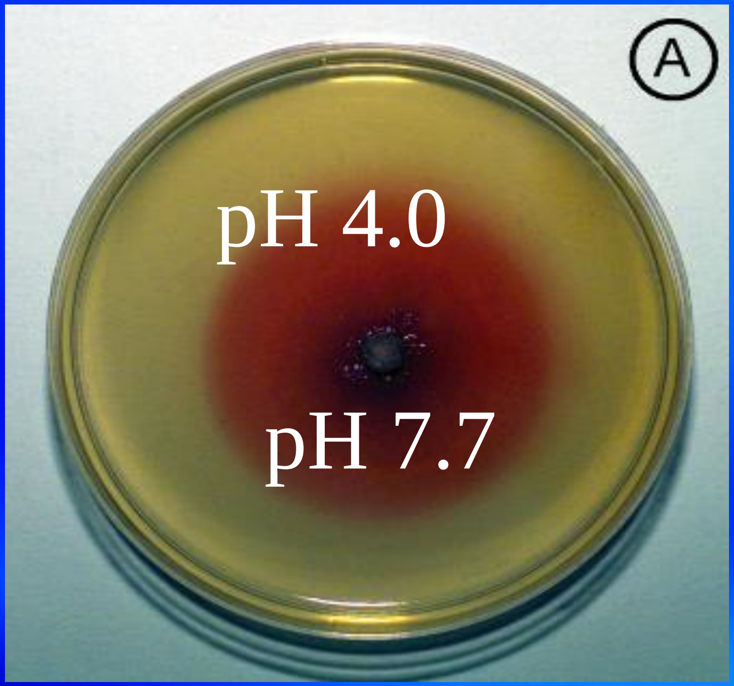
Pectate lyase
inducing media at pH 6.0

The pH of the host peel modulates the delivery and expression of pectolytic enzymes for colonization

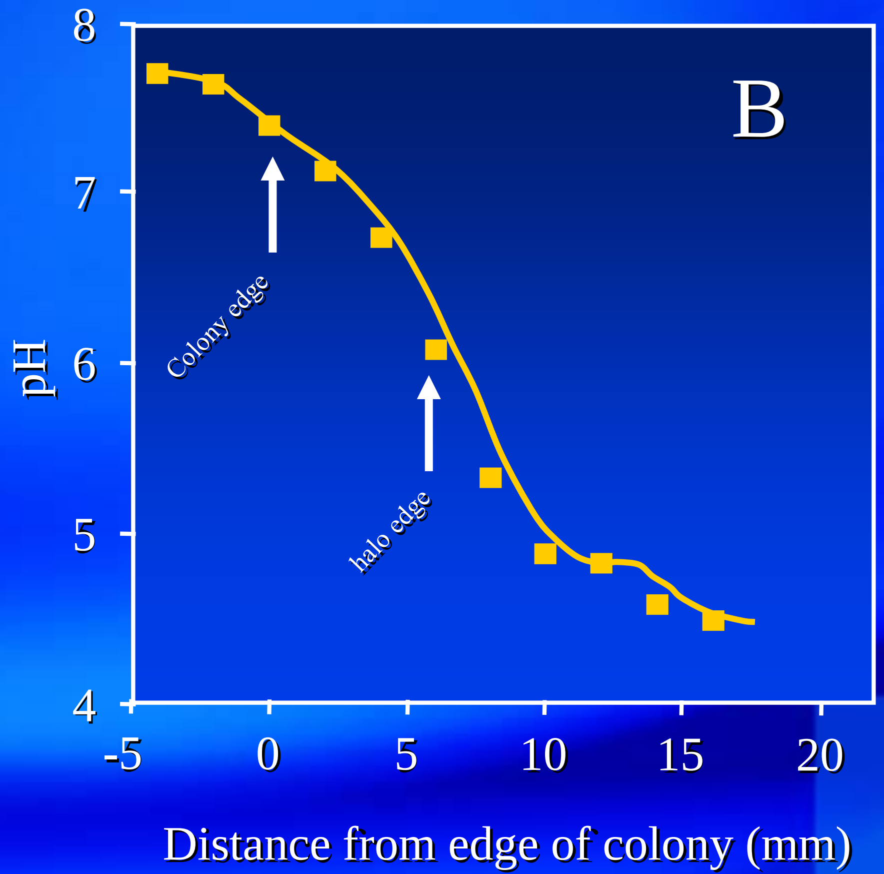
The second question:

Can the pathogen modulate the expression of it’s pathogenicity genes to enhance its colonization?

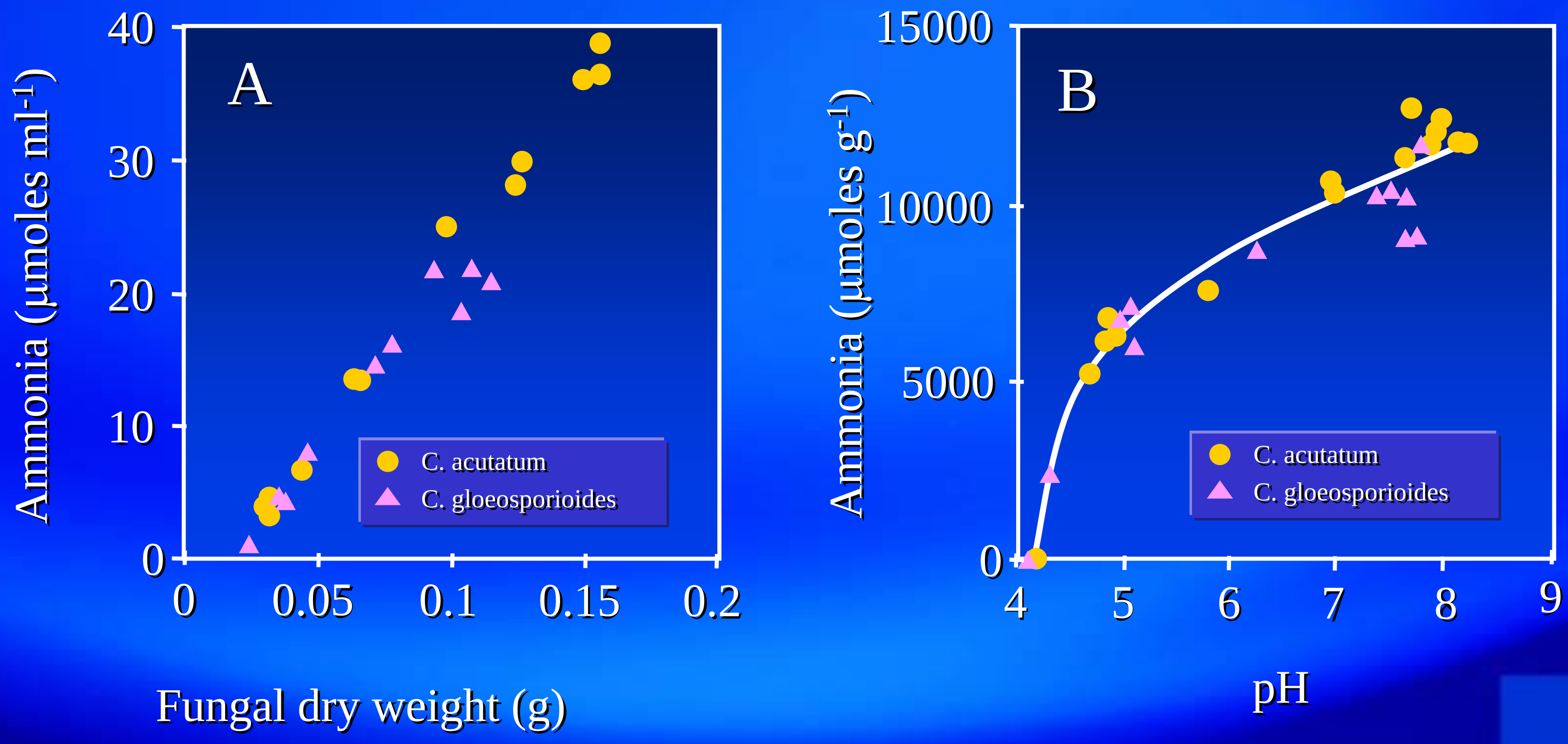
Differences in pH values induced by *C. gloeosporioides* in YE media at pH 4.0



With Alizarin Red

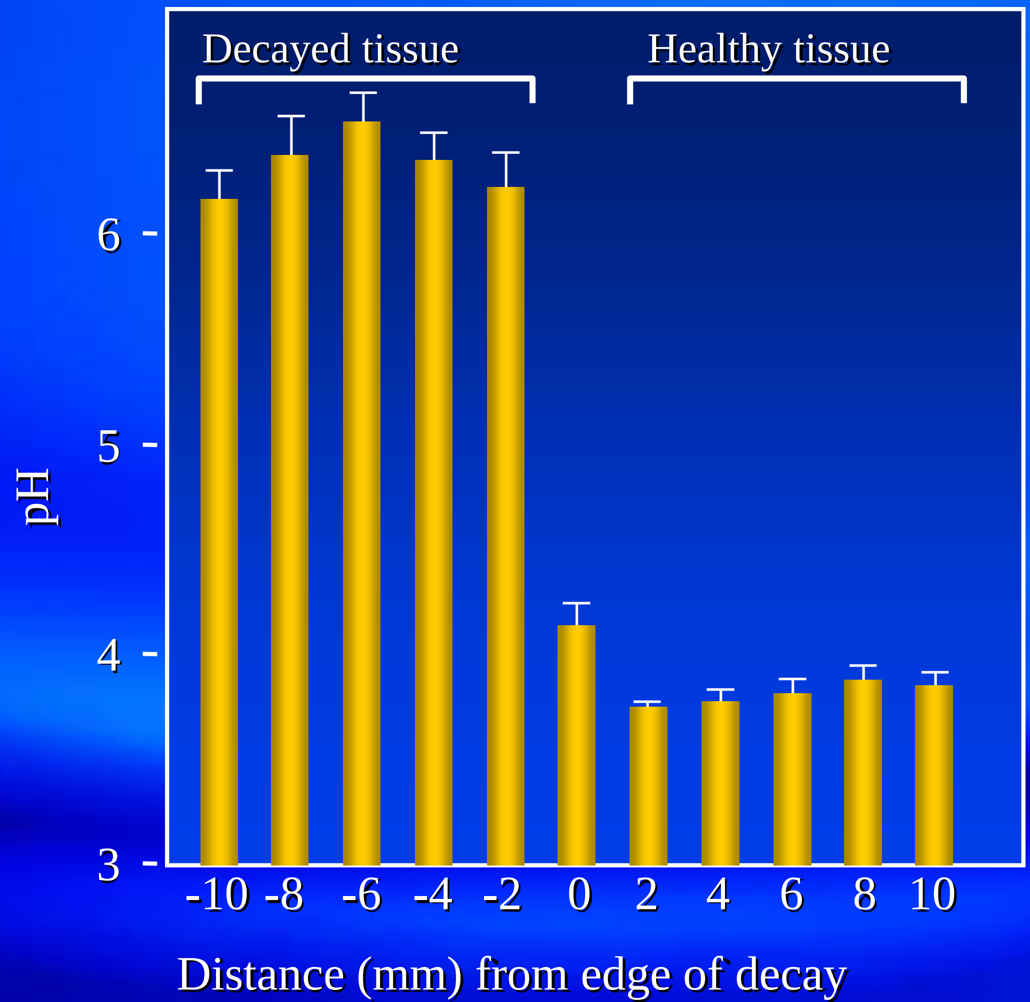


Changes in pH and ammonia accumulation induced by *C. gloeosporioides* in acidified YE media to pH 4.0

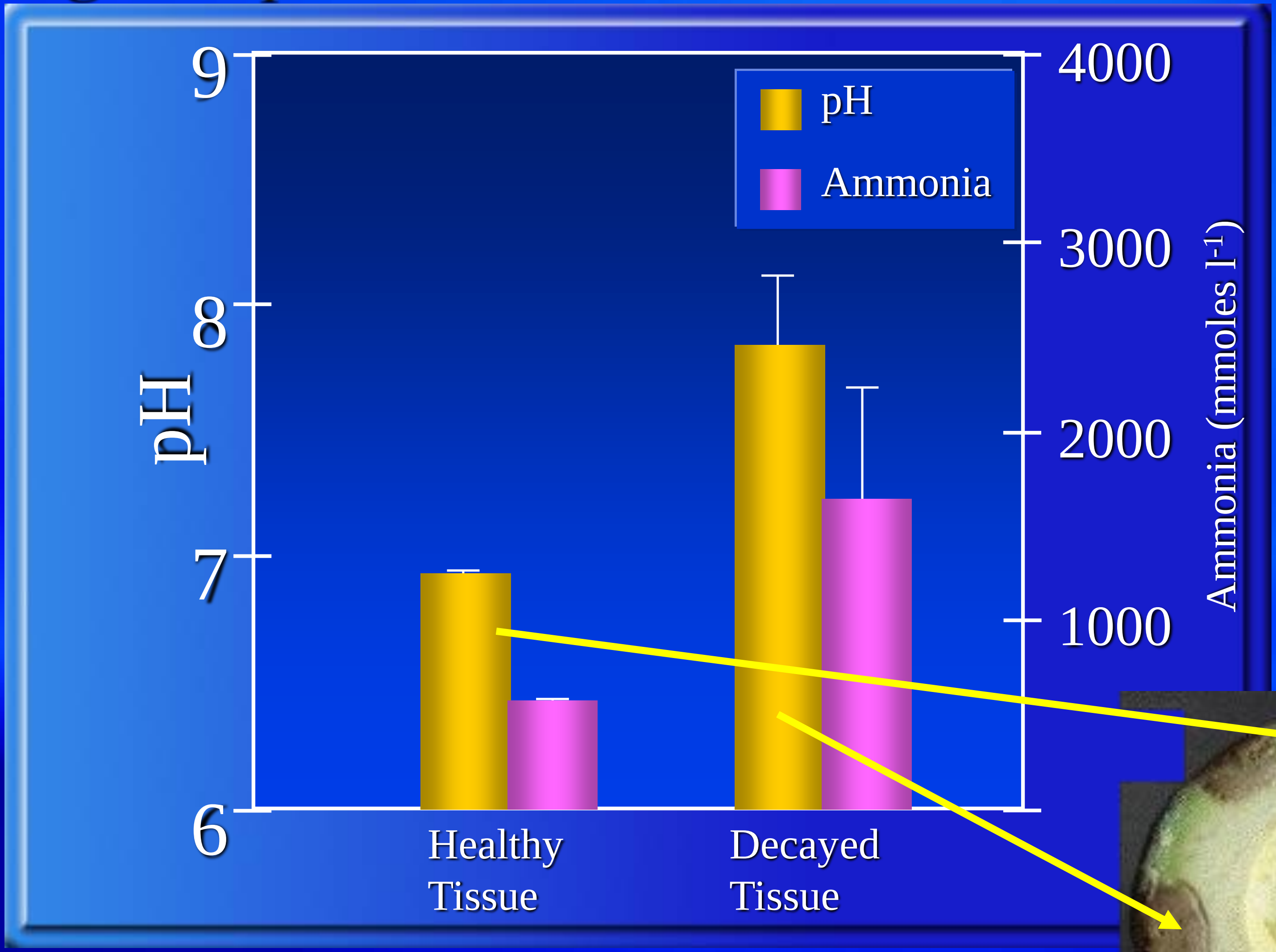


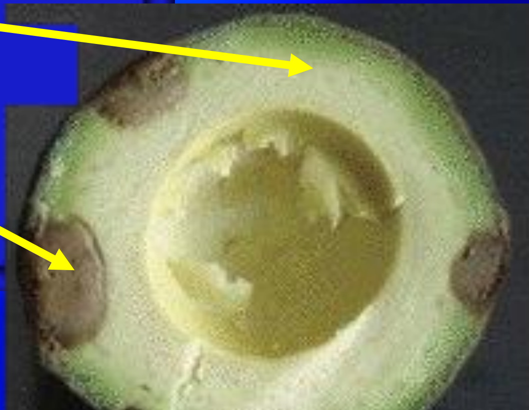
Prusky et al., 1991 MPMI

pH values at different distance of the leading edge of the decay caused by *C. acutatm* in apple fruits

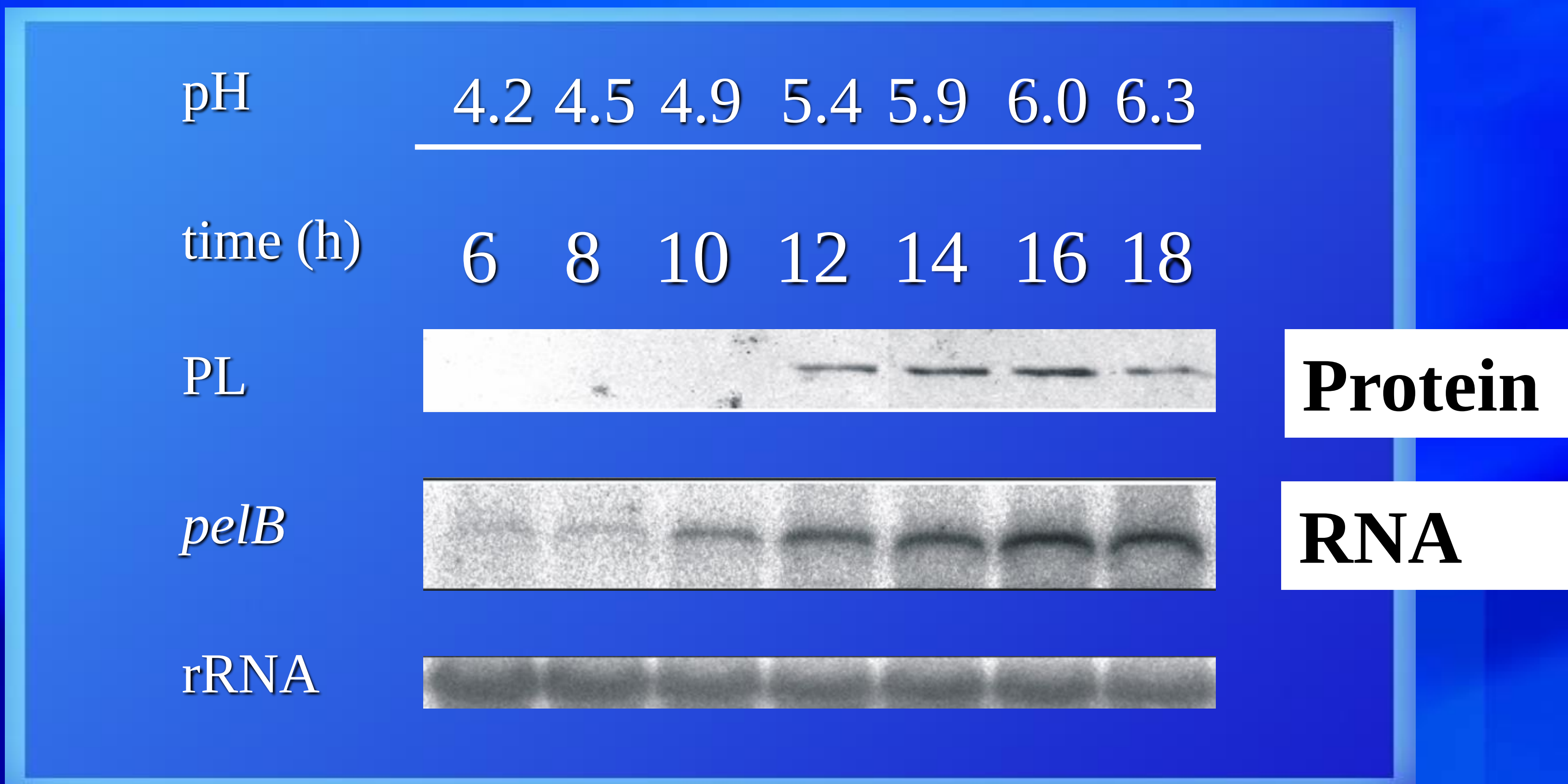


pH changes and ammonia accumulation by *C. gloeosporioides* in avocado fruits cv. Hass





Transcriptional activation of *pelB* and PL secretion by *C. gloeosporioides* as a function of pH



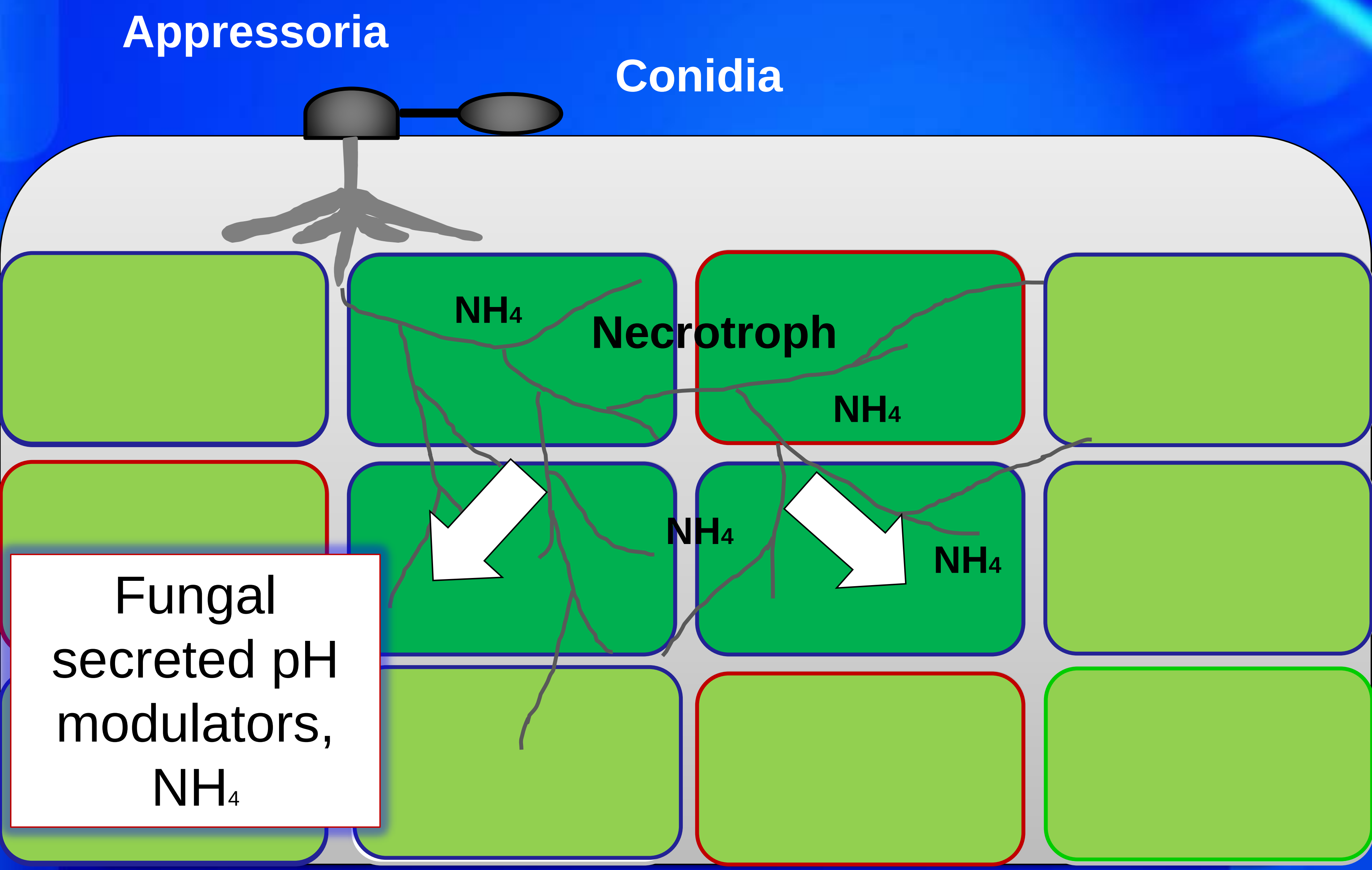
Summary of factors of pathogenicity

1. The pathogen produce pectinases (PME, PG and PelB)
2. The pathogen produces also cellulases
3. The activation of enzymes are inductive by the presence of subtrates
4. The activation of genes and enzymes are pH dependent

Case studies

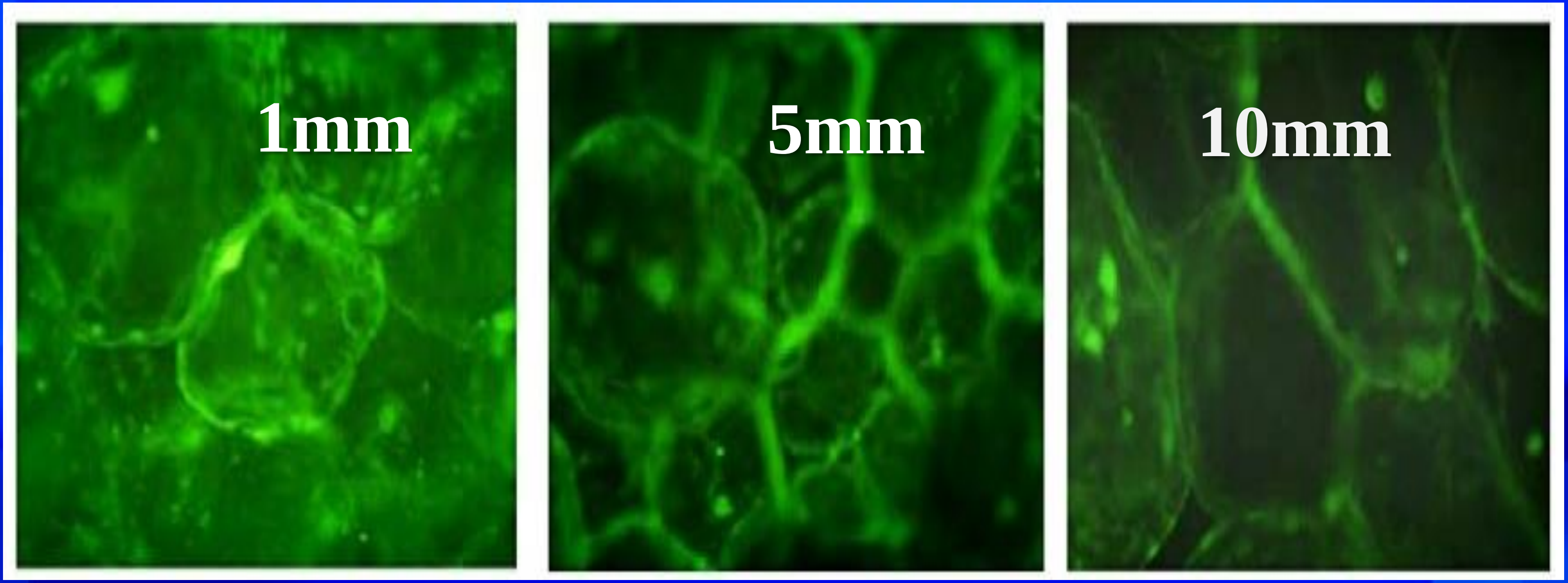
Alkalinization of the host tissue

Alkalization by Colletotrichum in ripe fruits



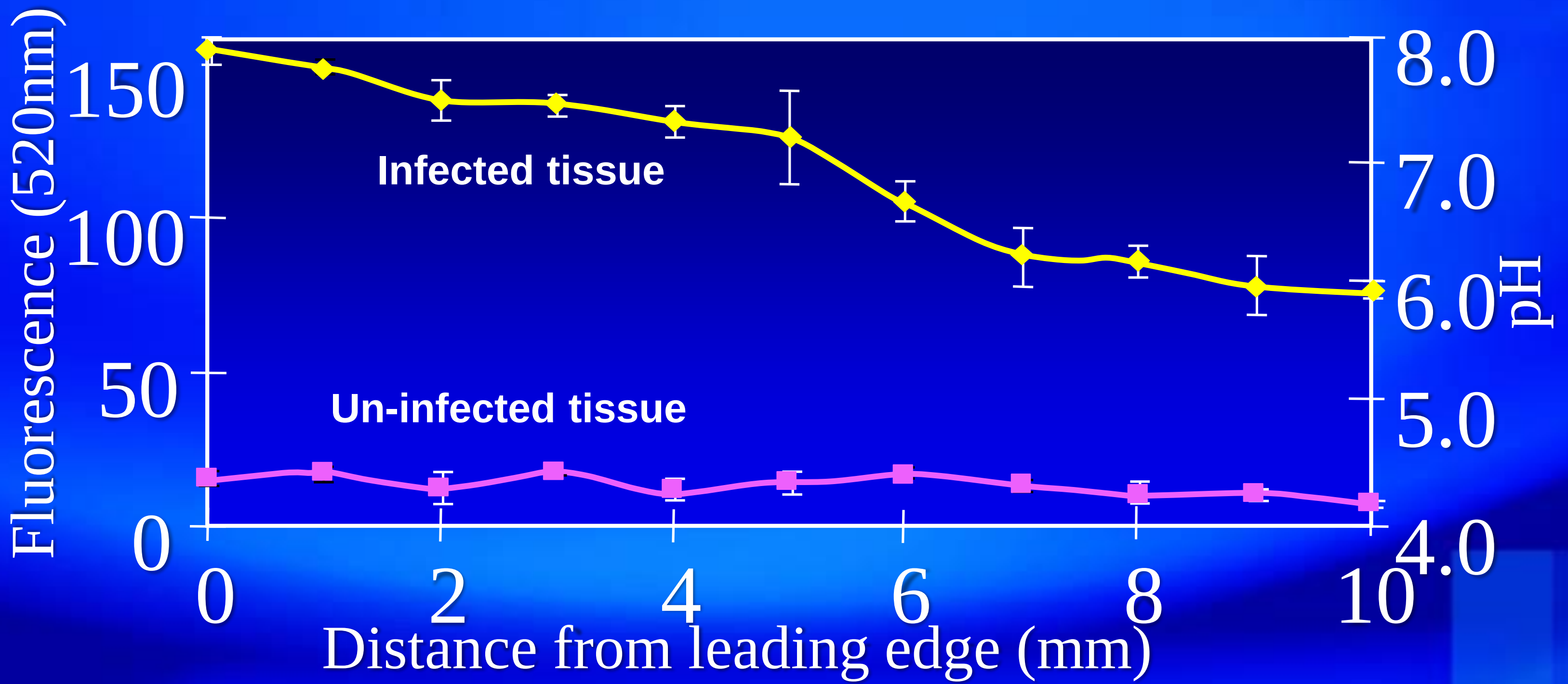
Local pH detected with BCECF in tomato fruit inoculated with *C. coccodes*

Distance from leading edge (mm)



2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF), fluorescent dye to measure intracellular pH

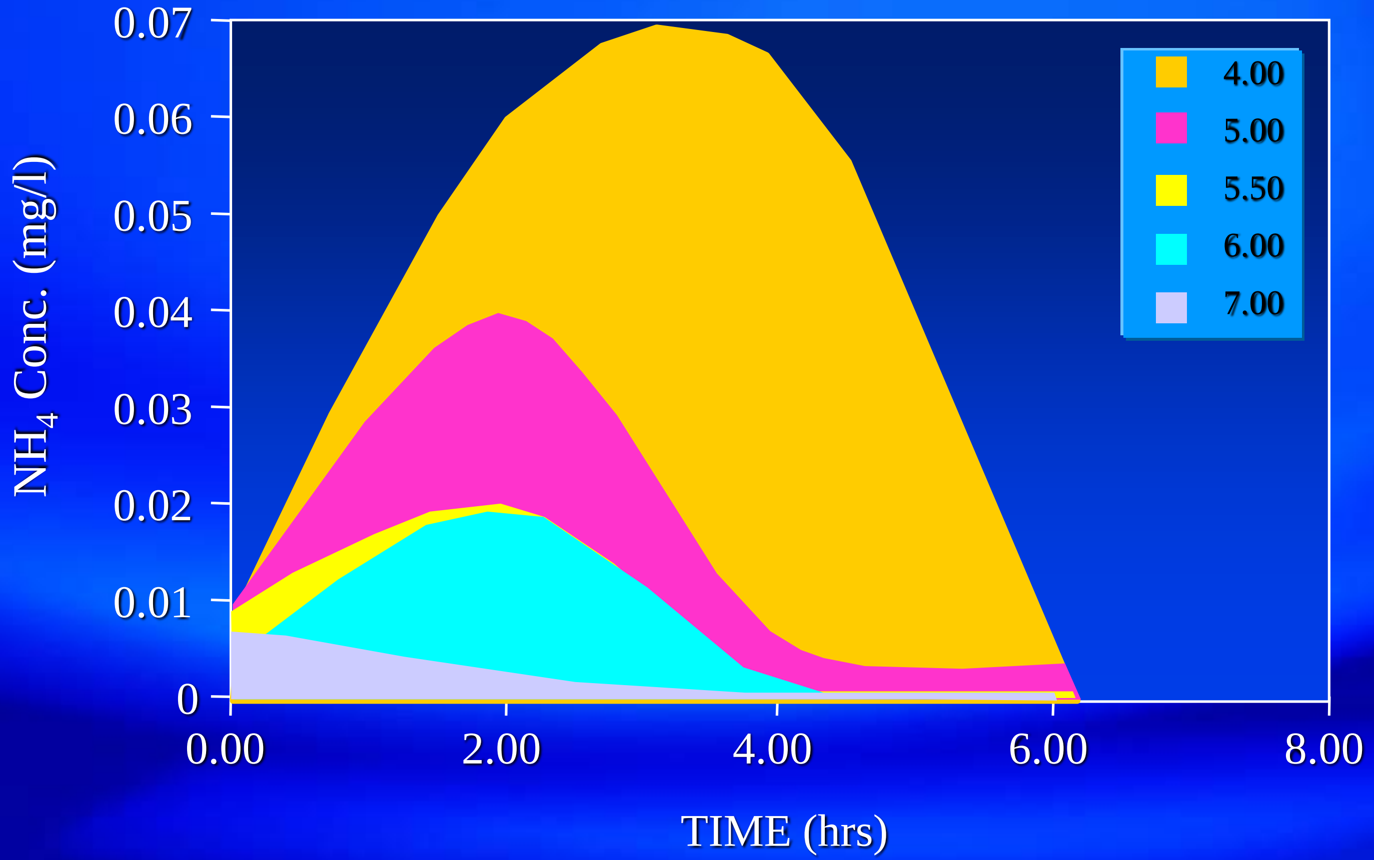
Local pH environment in tomato fruit at the leading edge of the decay caused by *C. coccodes*



Alkan et al, MPMI, 2008

What is the signal that activates ammonia secretion?

Effect of pH on the inducing the secretion and accumulation of NH_4 by *C. gloeosporioides*?



Kramer-Haimovitz et al. AEM, 2005

What is the importance of pH modulation of the host tissue?

Secretion and accumulation of pectate lyase by *C. gloeosporioides* as a function of pH

pH	4.2	4.5	4.9	5.4	5.9	6.0	6.3
time (h)	6	8	10	12	14	16	18

PL

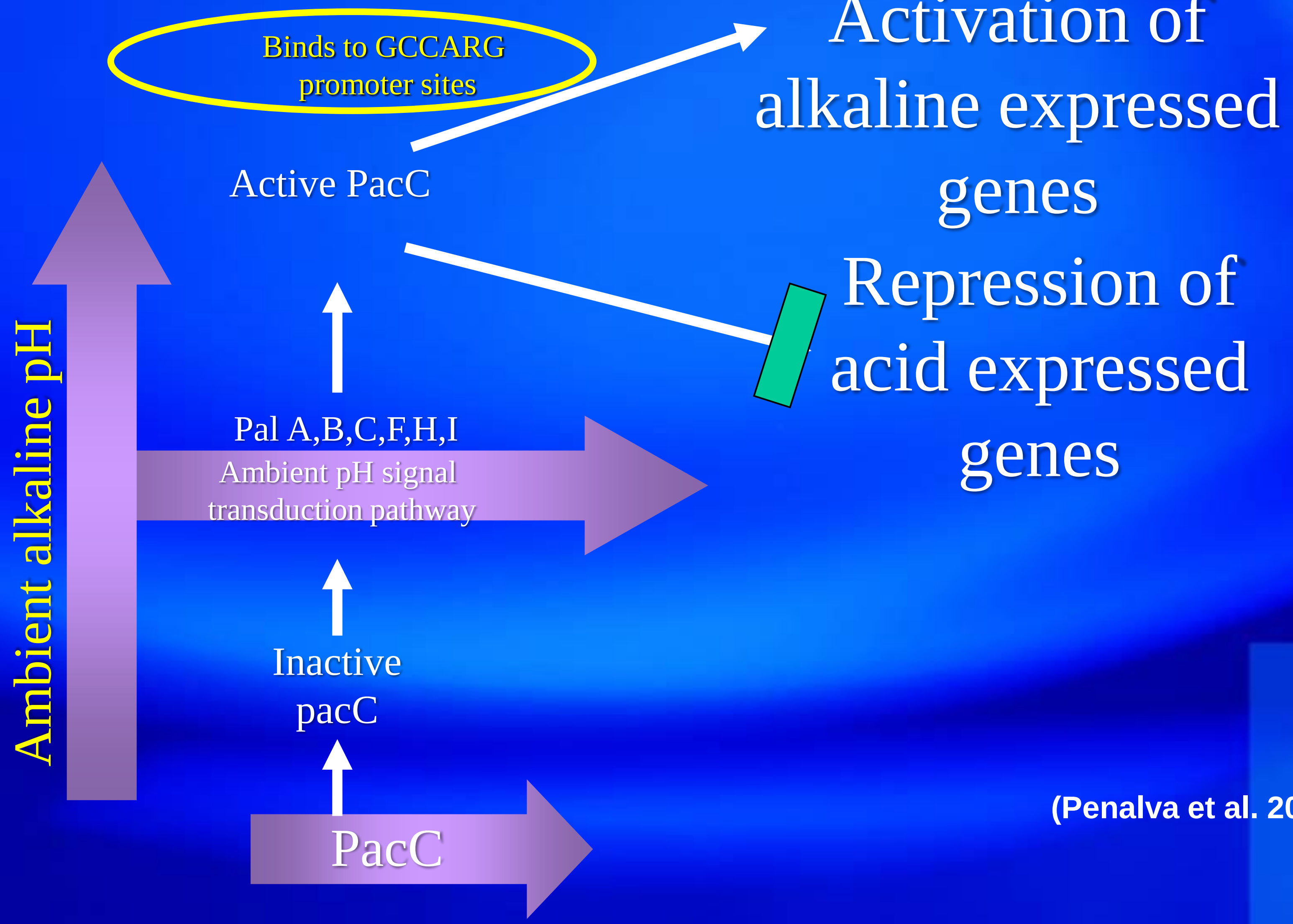
Increased maceration capabilities

pH modulation of the environment

enables the pathogen the "selection" of specific virulence factors needed for the particular host

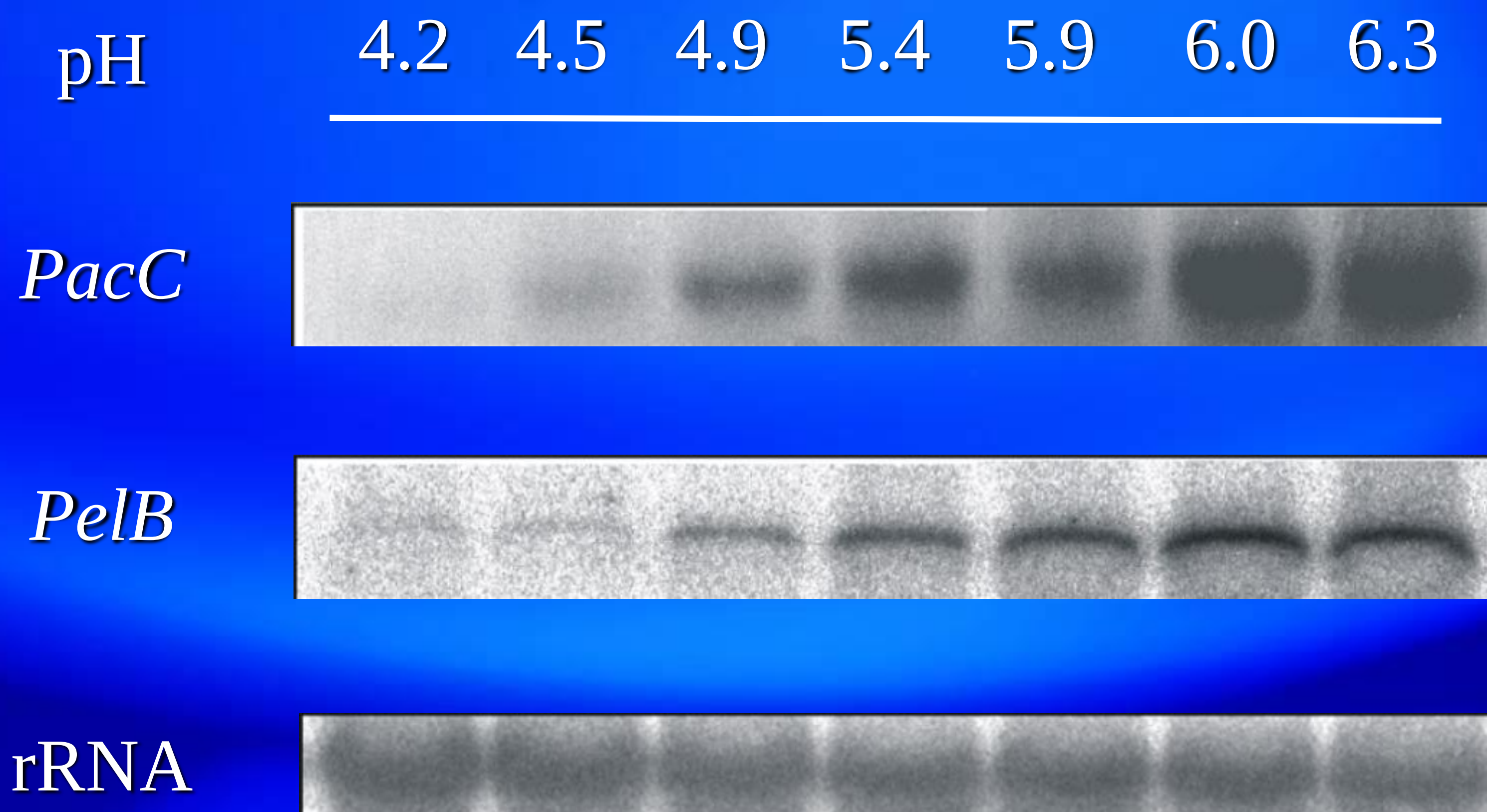
How does alkalization of the tissue modulates gene expression?

The modulation of genes at alkaline pH by the transcription factor *pacC*



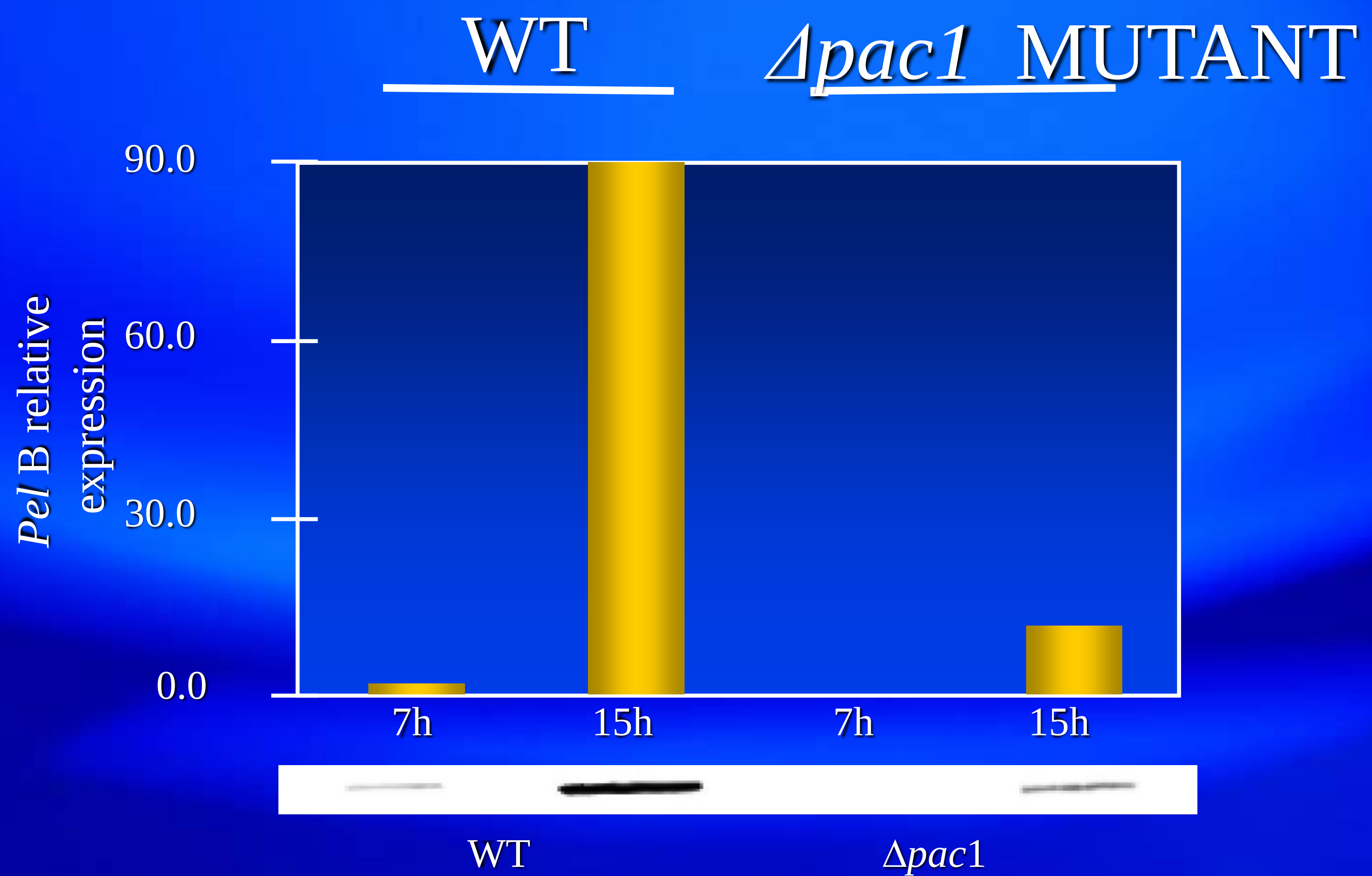
(Penalva et al. 2013)

Transcriptional activation of *PacC* and *pelB* from *C. gloeosporioides* as a function of pH



Knock out of *pac*C, the transcription factor that modulate gene expression at alkaline pH, down-regulated PL expression and reduced pathogenicity

Transcriptional activation of *pelB* and PL secretion detected by Western Blots of the WT *C. gloeosporioides* and Δ*pac1* mutant at pH 6.0



Effect of knock out of genes on reduced pathogenicity by *C. gloeosporioides*

	Reduced Colonization (% control)
*pel*B	25-30
*pac*C	70-80

Effect of *PacC* knock out on decay development

WT

Δ*pac*1

Miyara et al., 2008. MPMI

What is the purpose of the pH modulation?

- Specific activation of fungal genes
- Specific production pectolytic enzymes
- Enhance activity of the pectolytic enzymes

How is this carried out?

Activation of biochemical process regulating the production of

- ammonia

Second case studies:

pH regulators by acidification of the tissue

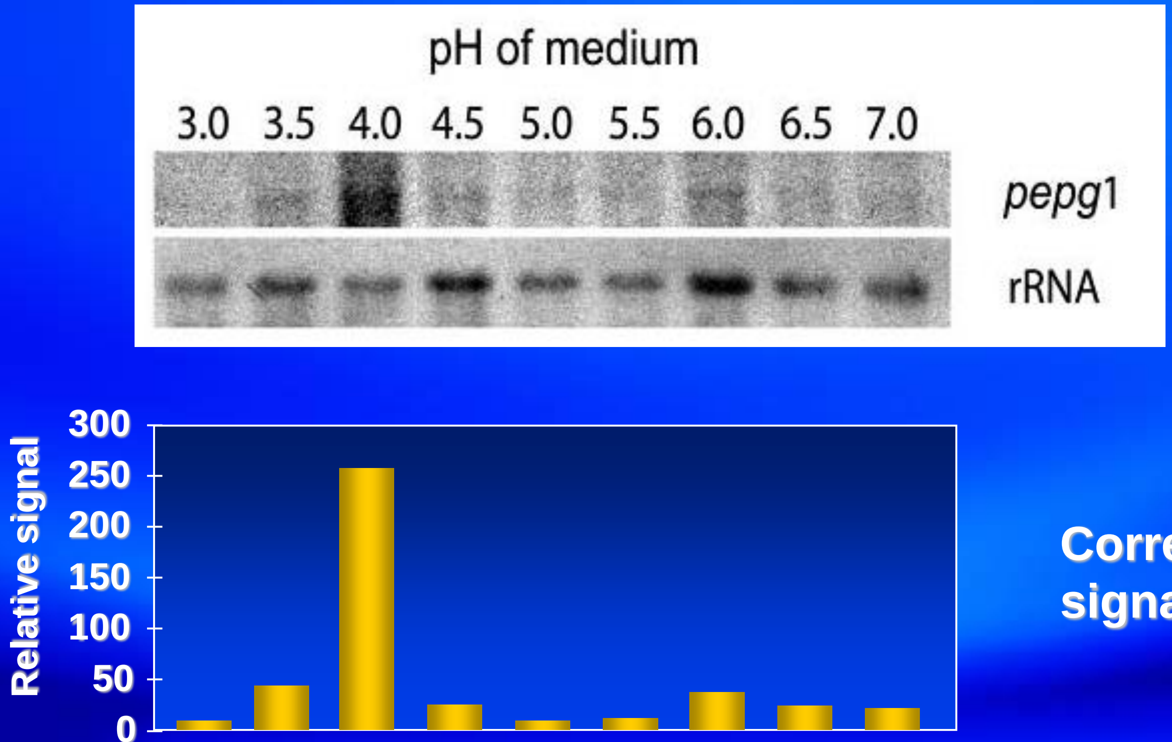
The *Penicillium* case

pH of healthy and decayed apple tissue inoculated by *P. expansum*

Apple cultivars	Healthy	Decayed
Fuji	4.57 a	3.92 b
Gala	4.35 a	3.91 b
Red Delicious	4.39 a	4.01 b
Granny Smith	4.04 a	3.65 b
Golden Delicious	4.60 a	3.80 b

How does acidification modulate virulence of *Penicillium*

Polyglacturonase, *pepg*1 transcript accumulation of *P. expansum* in response to different ambient pH conditions



Corrected *pepg*1 signals

What is the mechanism of acidification of host tissue

Accumulation of organic acid in decayed apples by *P. expansum*

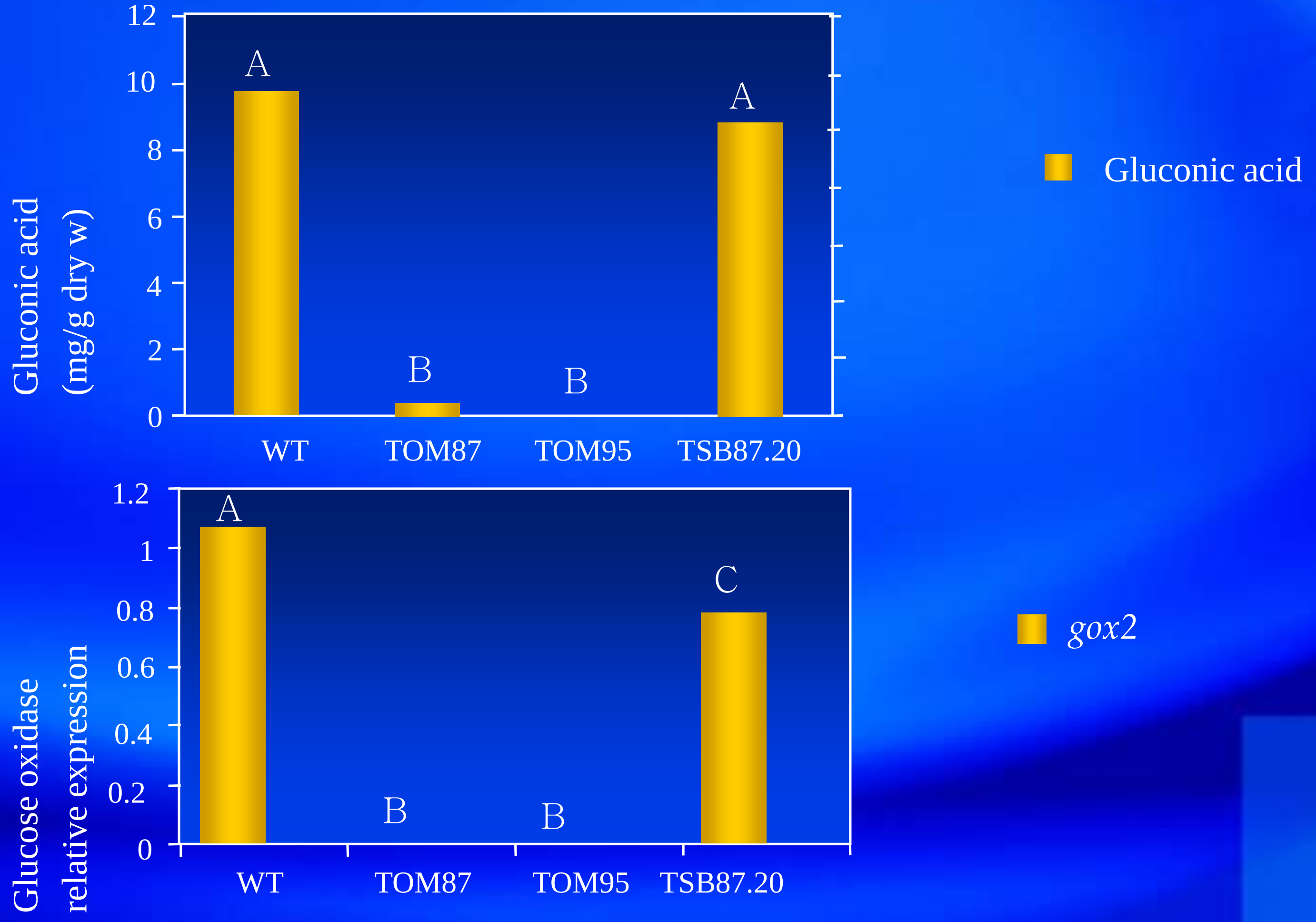
Organic acid (μg/gr fw)	Healthy	Decayed
Gluconic	182±39	2334±814
Citric	193±53	1410±211
Malic	4020±370	4380±680
Fumaric	1.4±0.3	15.8±1.8

Glucose oxidase

ß-D-glucose + ½O_2 $\rightarrow$ gluconic acid + H_2O_2

***gox*1 and *gox*2**

Disruption of *gox*2 reduced *gox*2 relative expression and gluconic acid accumulation



What is the purpose of the pH modulation?

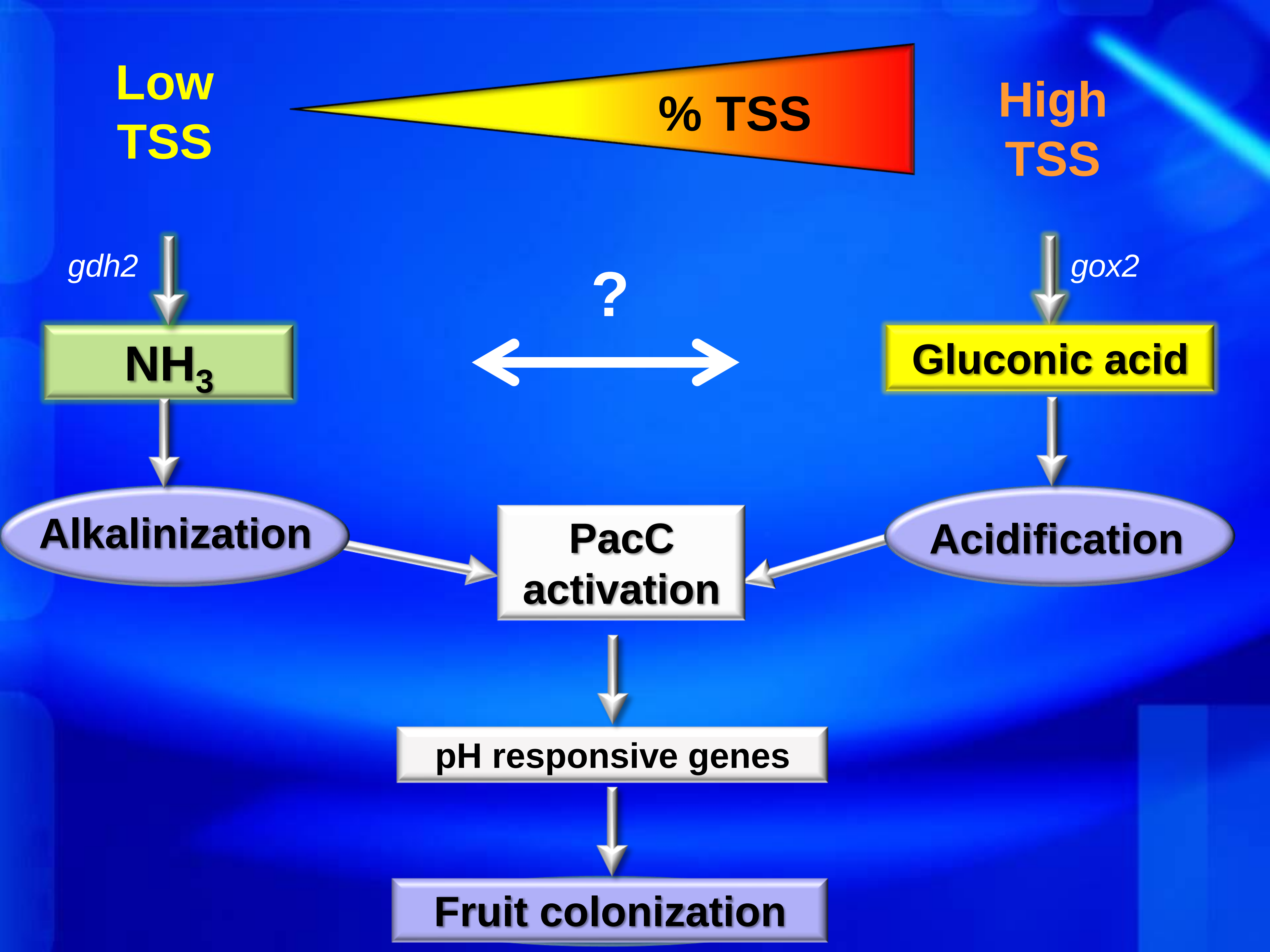
- Specific activation of fungal genes
- Specific production pectolytic enzymes
- Enhance activity of the pectolytic enzymes

How is this carried out?

Activation of biochemical process regulating the production of

- Gluconic acid and the acidification of the host tissue

How is this mechanism activated in vivo



Low TSS
% TSS
High TSS
gdh2
gox2
?
NH3
Gluconic acid
Alkalinization
PacC activation
Acidification
pH responsive genes
Fruit colonization

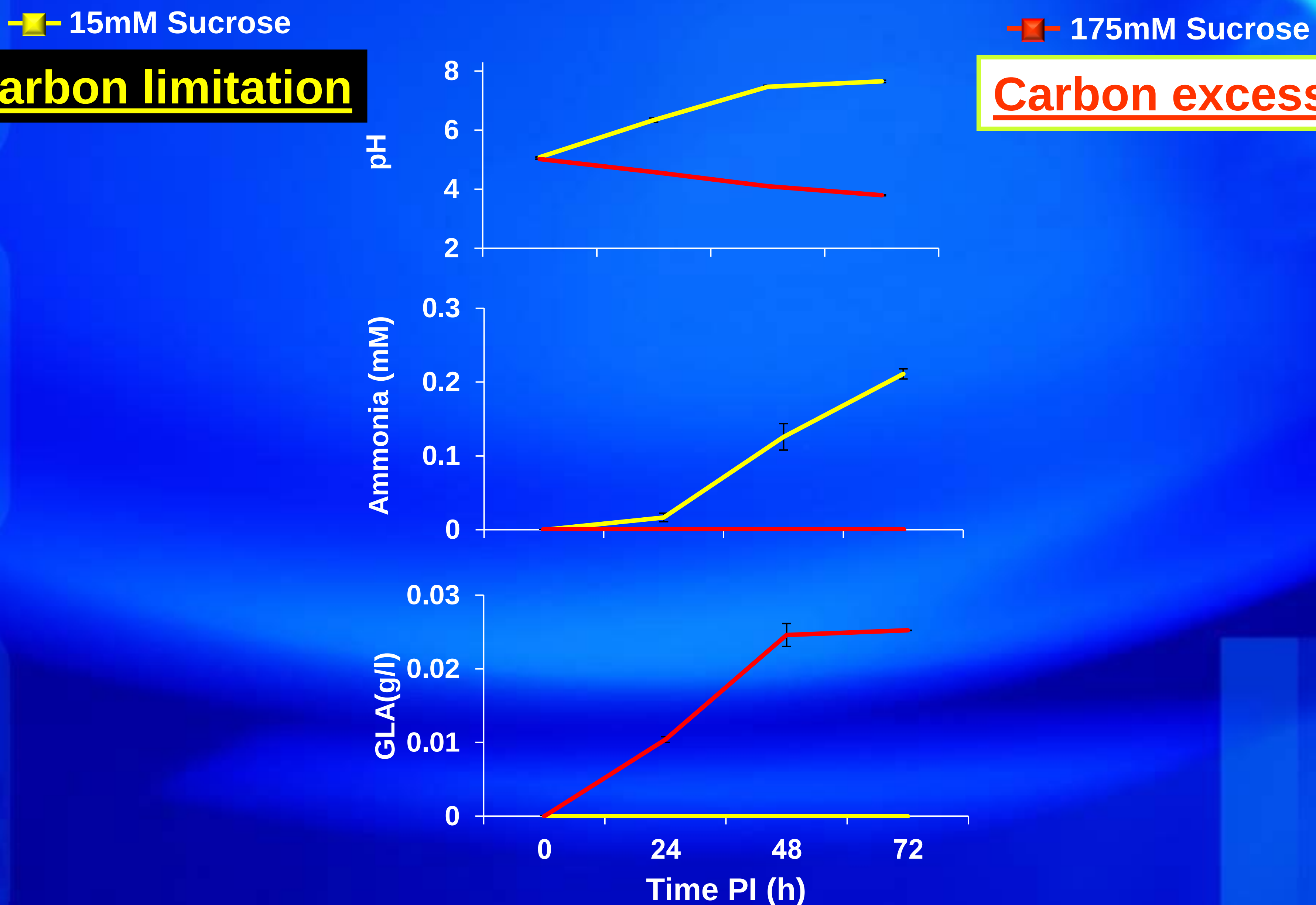
Biochemical factors regulating the synthesis of ammonia or organic acid

Carbon regulation

Effect sucrose concentration on pH modulation by *C. gloeosporioides*

Carbon limitation

Carbon excess



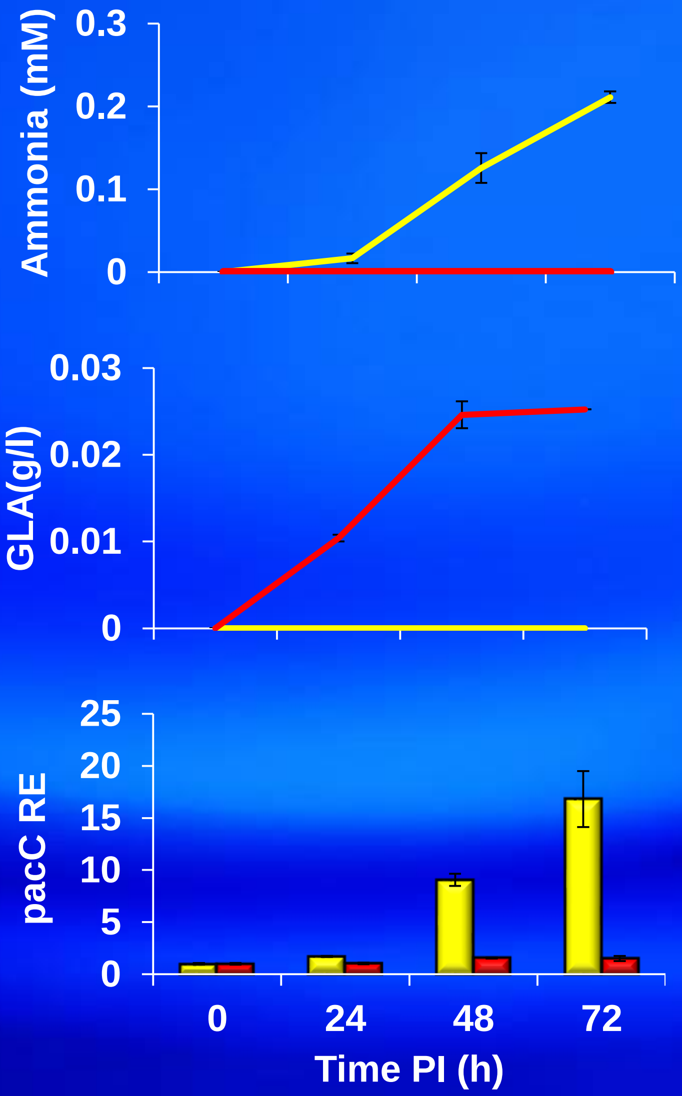
Effect sucrose concentration on pacC expression by *C. gloeosporioides*

15mM Sucrose

175mM Sucrose

Carbon limitation

Carbon excess



Is this mechanism occuring in the acidifying postharvest pathogen *Penicillium expansum*?

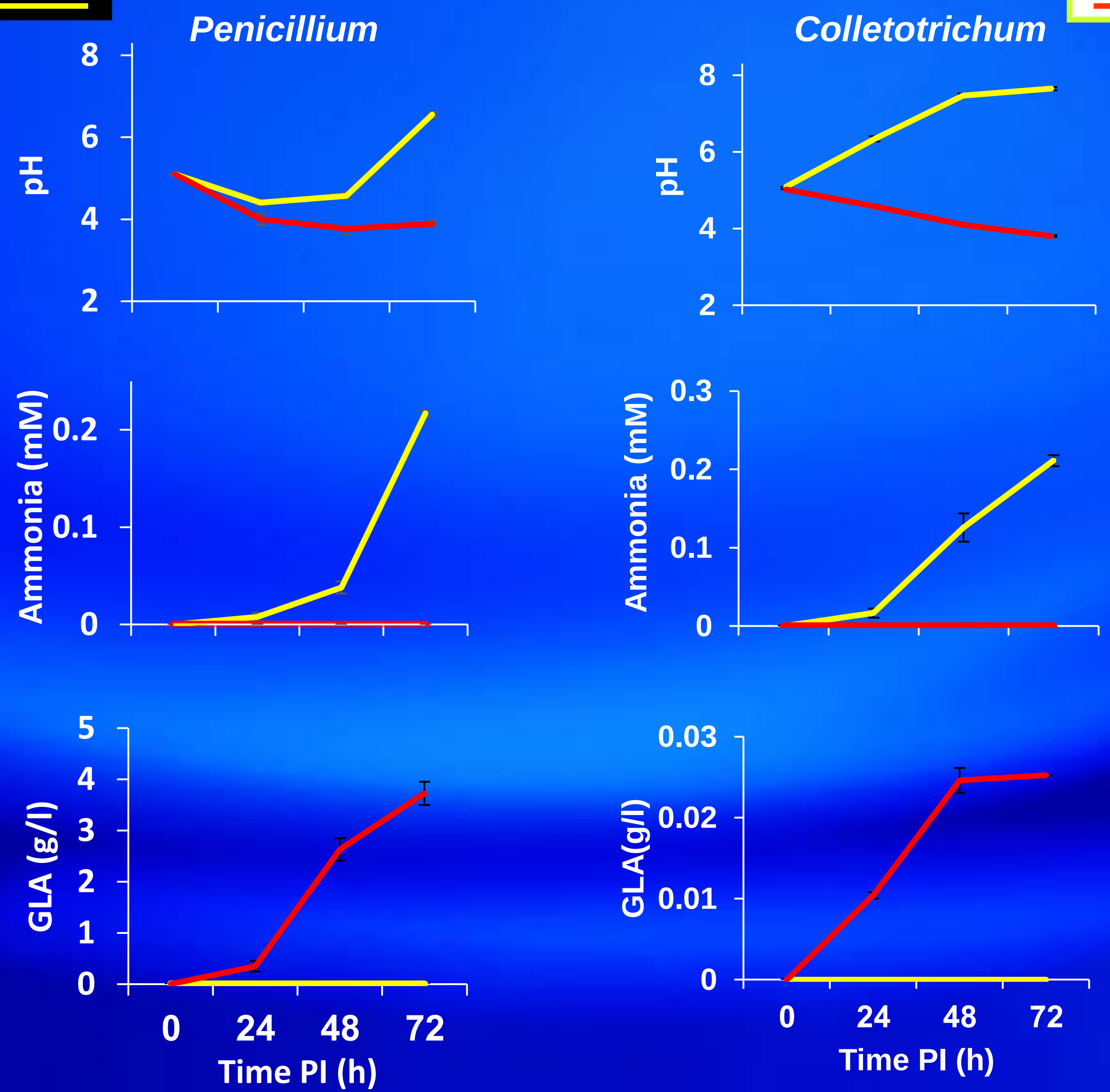
Effect of carbon on pH regulation by *Colletotrichum* and *Penicillium*

15mM Sucrose

175mM Sucrose

Carbon limitation

Carbon excess

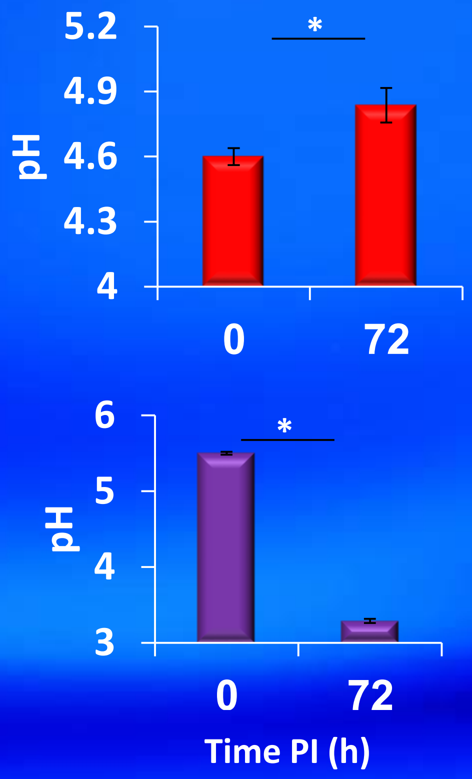


Do we have a sugar response present in fruits?

Effects of TSS on pH regulation of fruits and pathogenicity of *Colletotrichum*

Tomato
TSS 6.0

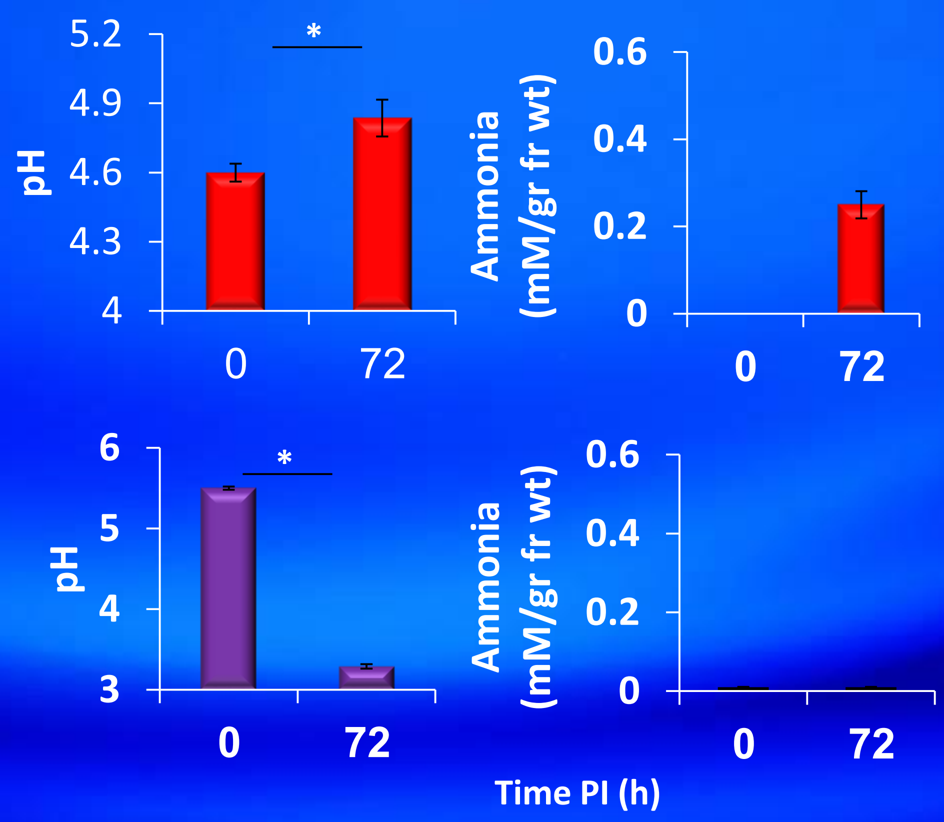
Plum
TSS 14.3



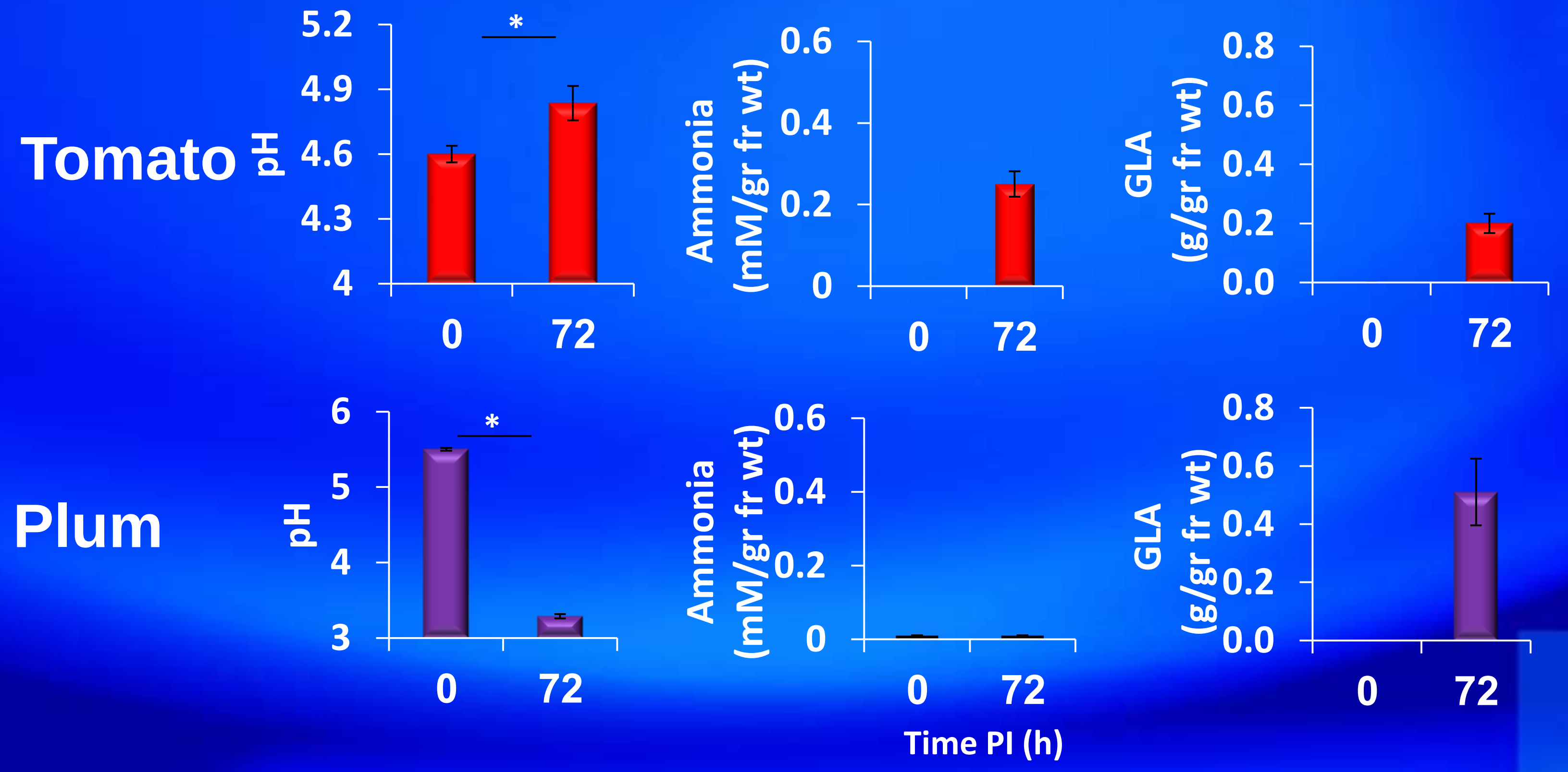
Effects of TSS on pH and ammonia regulation of fruits and pathogenicity of *Colletotrichum*

Tomato

Plum



Effects of TSS on pH, ammonia and GLA regulation of fruits and pathogenicity of *Colletotrichum*

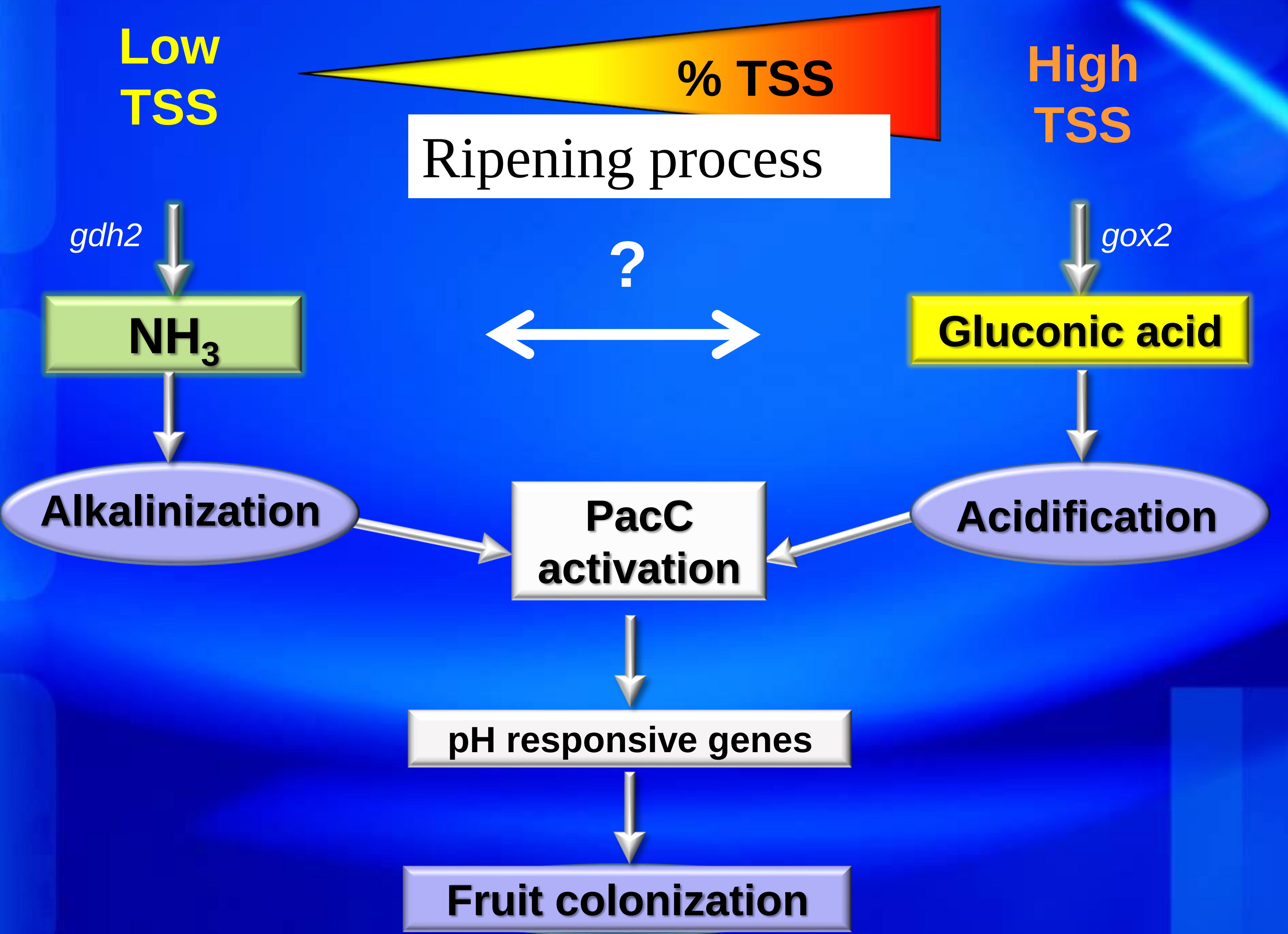


pH modulation by pathogens (2016)

	Alkalinizers and Acidifiers
Colletotrichum	Ammonia and GLA
Fusarium	Ammonia and GLA
Penicillium	Ammonia and GLA
Sclerotinia	Ammonia and GLA

The fruit carbon content regulate the mechanism of fungal pH regulation in fruits

Fangcheng Bi, et al. 2016 Carbon regulation of environmental pH by secreted small molecule effectors modulates pathogenicity in fungi. In Press: Molecular Plant Pathology



Low TSS
% TSS
High TSS
Ripening process
gdh2
?
gox2
NH_3
Gluconic acid
Alkalinization
PacC activation
Acidification
pH responsive genes
Fruit colonization

Summary

- Present results indicate the close relation between the accumulation of gluconic acid and pathogenicity of *P. expansum*
- Differential regulation of *gox2* might ensure tissue acidification while the contribution of *gox*1 is not significant
- Initial pH is the major factor affecting glucose oxidase expression and gluconic acid production

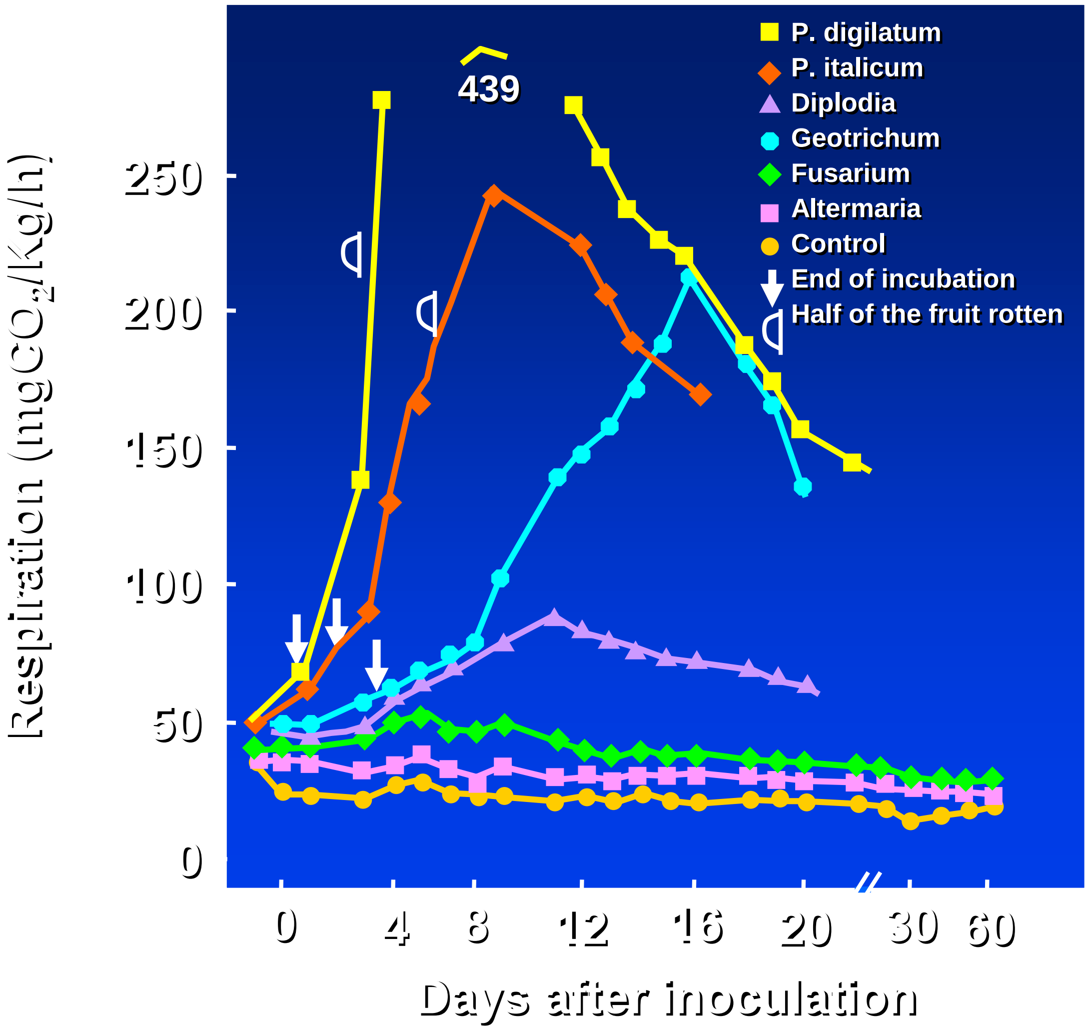
Summary of the three last lectures:

Mechanisms modulating pathogenicity in postharvest pathogens interactions

- Fungal pathogenicity is firstly determined by host resistance
- This response is mainly determined by the maturity and ripeness of the host
- The pathogen has an active function in the initiation of fungal attack by reacting to the host pH and secreting ammonia and/or organic acids leading to enhanced colonization

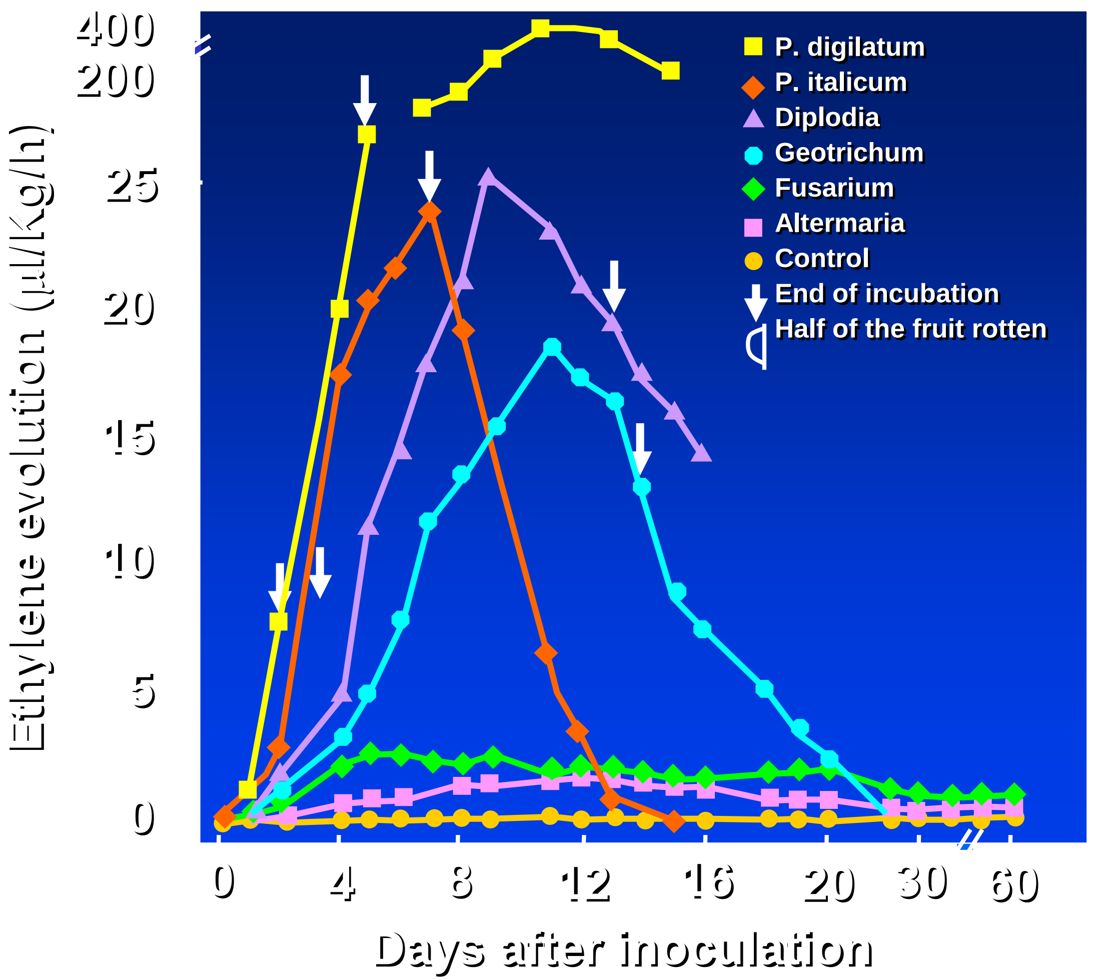
The physiological importance of fungal colonization on fruit physiology

Respiration rate of green lemon fruit inoculated with postharvest fungi



Reproduced from Schiffmann-Nadel, 1974

Ethylene evolution from green lemon fruit inoculated with postharvest fungi



Reproduced from Schiffmann-Nadel, 1974

Effect of fungal infection on softening and ripening

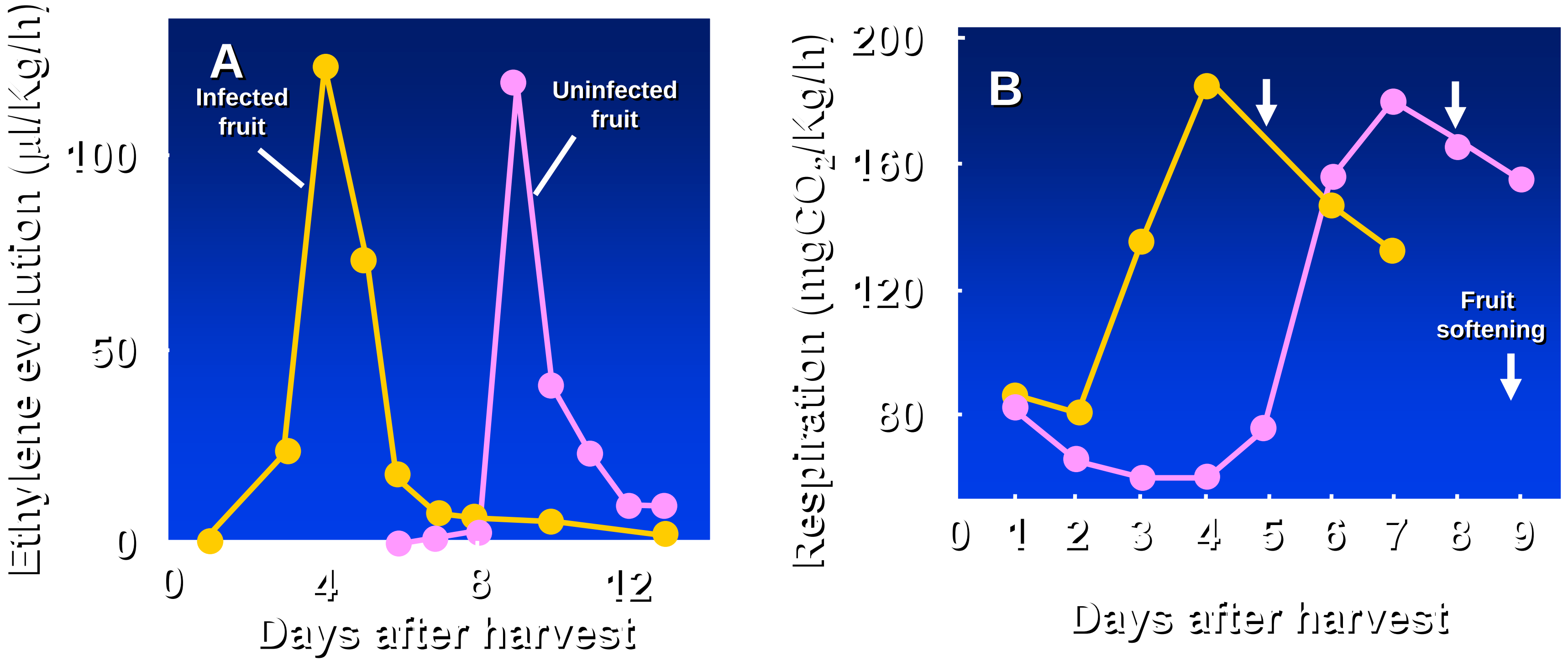


Fig. 20. Ethylene evolution (A) and respiration rates (B) of avocado fruit infected by *Fusarium solani* in comparison to uninfected fruit. (Reproduced from Zauberman and Schiffmann-Nadel, 1974 with permission of the American Phytopathological Society).