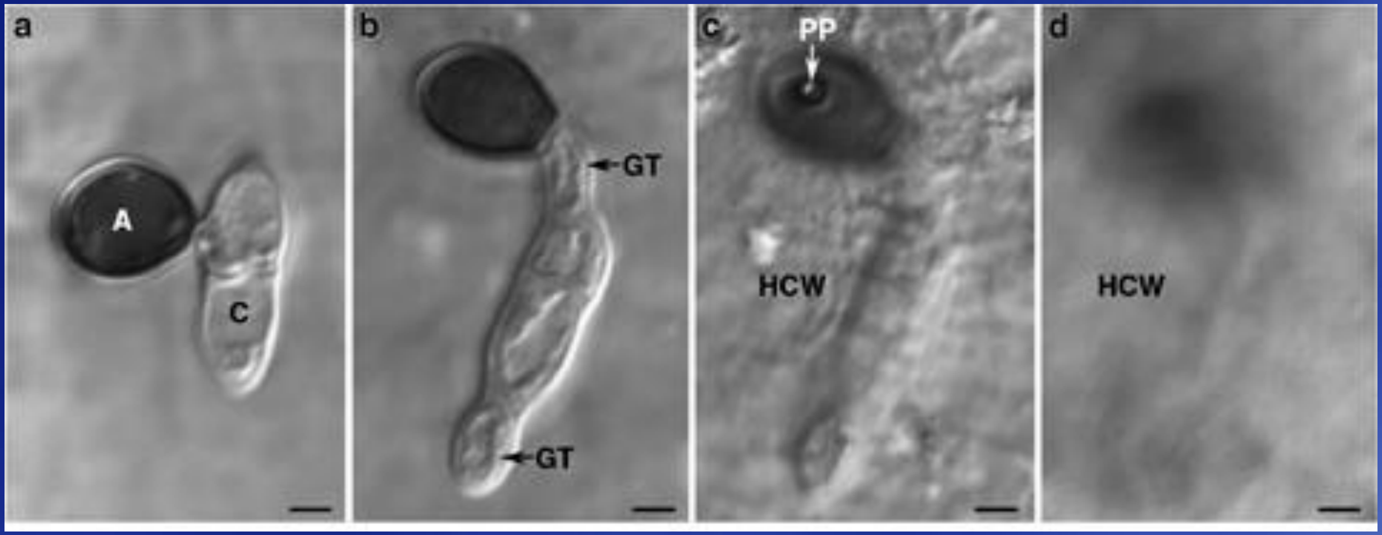
3rd Lecture

Quiescent infection and their activation during ripening

Following the penetration the next factor is:

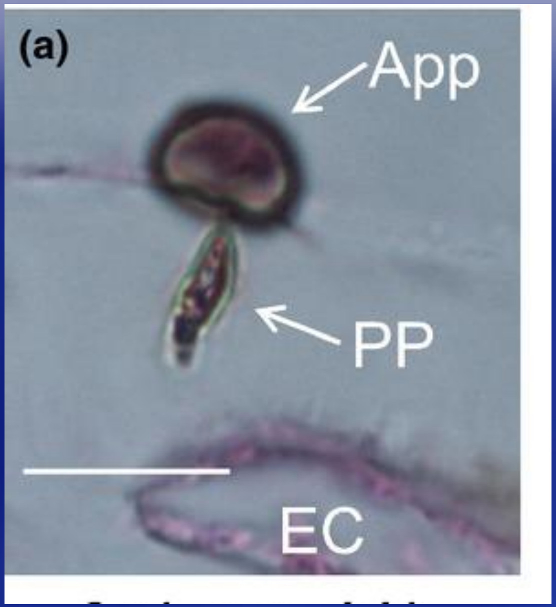
1. Activation of the quiescent infection
2. Colonization by the necrotrophic pathogen in the directly penetrated tissue

Micrographs showing conidia of *Colletotrichum acutatum* germinating on the surface of ripe blueberry fruit (cv. Jersey).



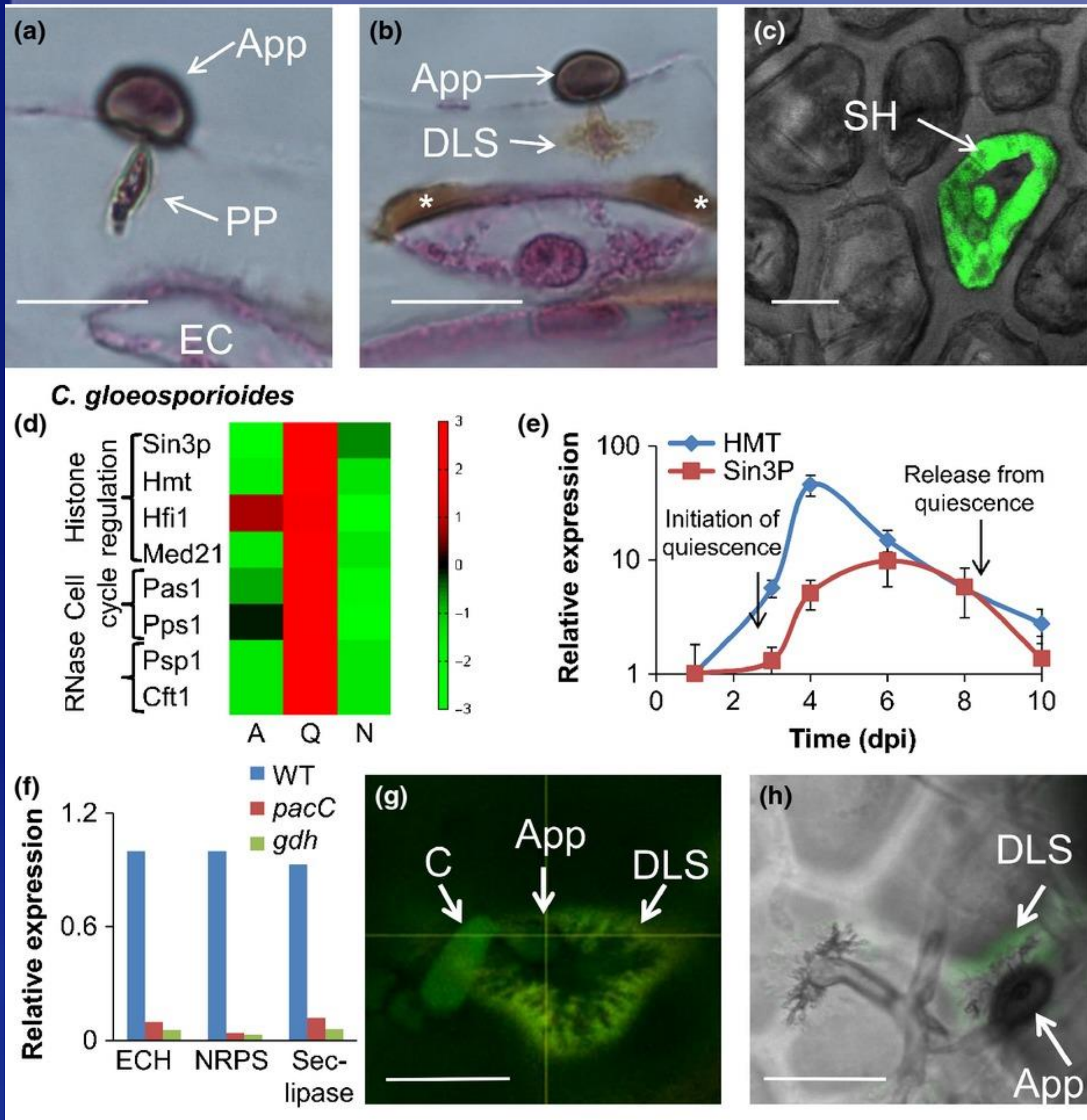
Appressoria were fully formed by 24 hpi and were either sessile (a) or subtended by a very short germ tube (b). Mature appressoria contained a penetration pore (PP) which was in contact with the host cell wall (HCW)

What happen to fungus during the quiescence?



During quiescence of *C. gloeosporioides* , 7903 fungal genes were expressed as compared with 10 450 and 11 446 in the appressoria and necrotrophic stages, respectively. Of these, 178 genes could be defined as quiescent-specific

Simultaneous transcriptome analysis of *Colletotrichum gloeosporioides* and tomato fruit pathosystem reveals novel fungal pathogenicity and fruit defense strategies



Histone modifiers (Sin3p, Hmt) and ATP-dependent chromatin remodeling complexes regulating DNA accessibility are crucial for gene activation repression.

Transcripts for histone modifiers were observed to be up-regulated specifically at the quiescent stage, including the fungal homologs
***Sin3p* 6 to 20-fold,**
***Hmt* 4 to 20-fold and**
***Hfi1* 2 to 15-fold**

Histone modifications are key epigenetic regulators that control chromatin structure and gene transcription, thereby impacting on various important cellular phenotypes.

Alkan et al., 2015. New Phytologist 205(2):801-15

- Active colonization of the tissue

Mechanism contributing to activation of quiescence and fungal colonization

1. The need of host nutritional factors for fungal initial fungal colonization
2. The decline of host preformed antifungal compounds during fruit ripening.
3. Occurrence of host inducible limiting compounds and that decline during fruit ripening.
4. Activation of fungal pathogenicity factors during ripening fruits.

The mechanism contributing to host resistance based in the lack of fungal colonization

Lack of host nutritional factors needed for fungal initial fungal colonization

The mechanism contributing to host resistance based in the lack of fungal colonization

Presence of host preformed antifungal compounds in the unripe fruit that decrease during fruit ripening

Presence of host preformed antifungal compounds

- The presence of antifungal compounds in unripe fruits suggest that they might be involved in fruit resistance
- However a close relation should be experimentally found between the antifungal compounds and fungal inhibition

Presence of host preformed antifungal compounds

- The Diene/Trien in avocado
- Tomatin (saponin) in tomato
- Resorcinols in mango
- Citral in citrus

Preformed antifungal compounds in avocado fruit

TRI-ENE

(1999)

(-cis- cis, trans)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene

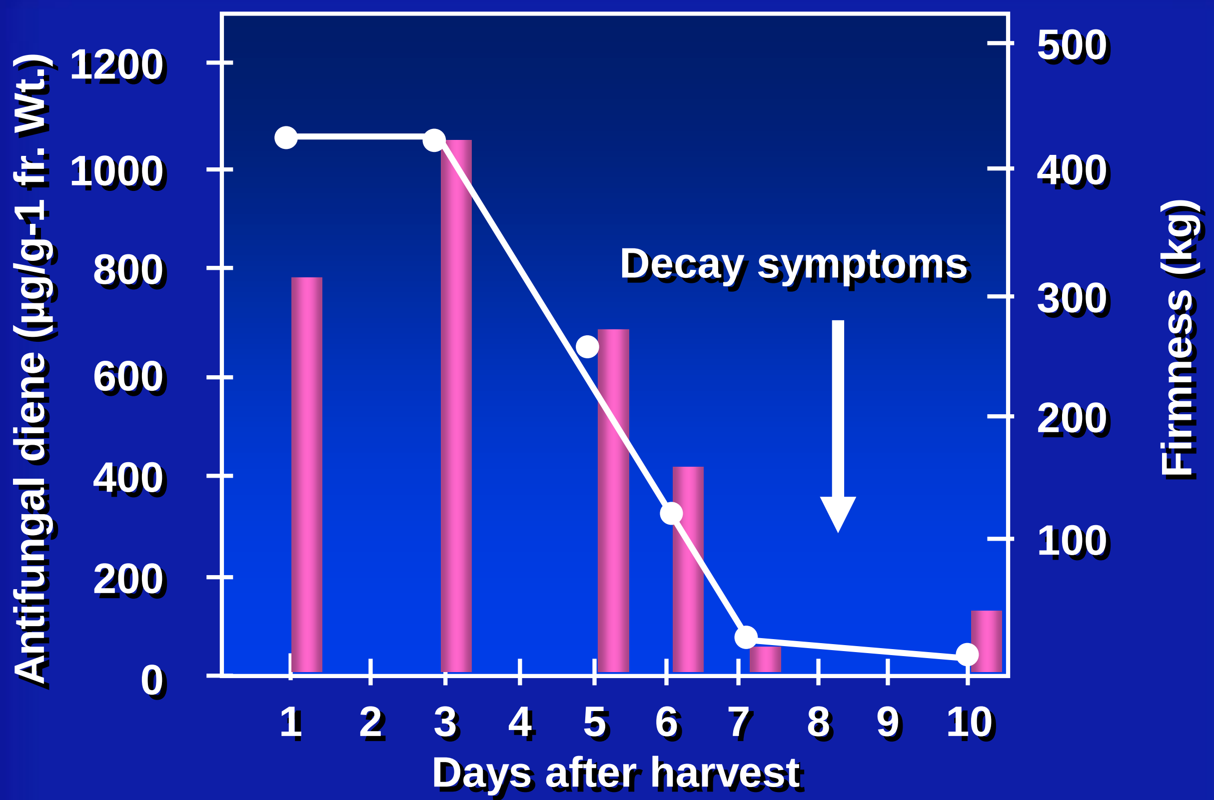
DI-ENE

(cis, cis)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (1981)

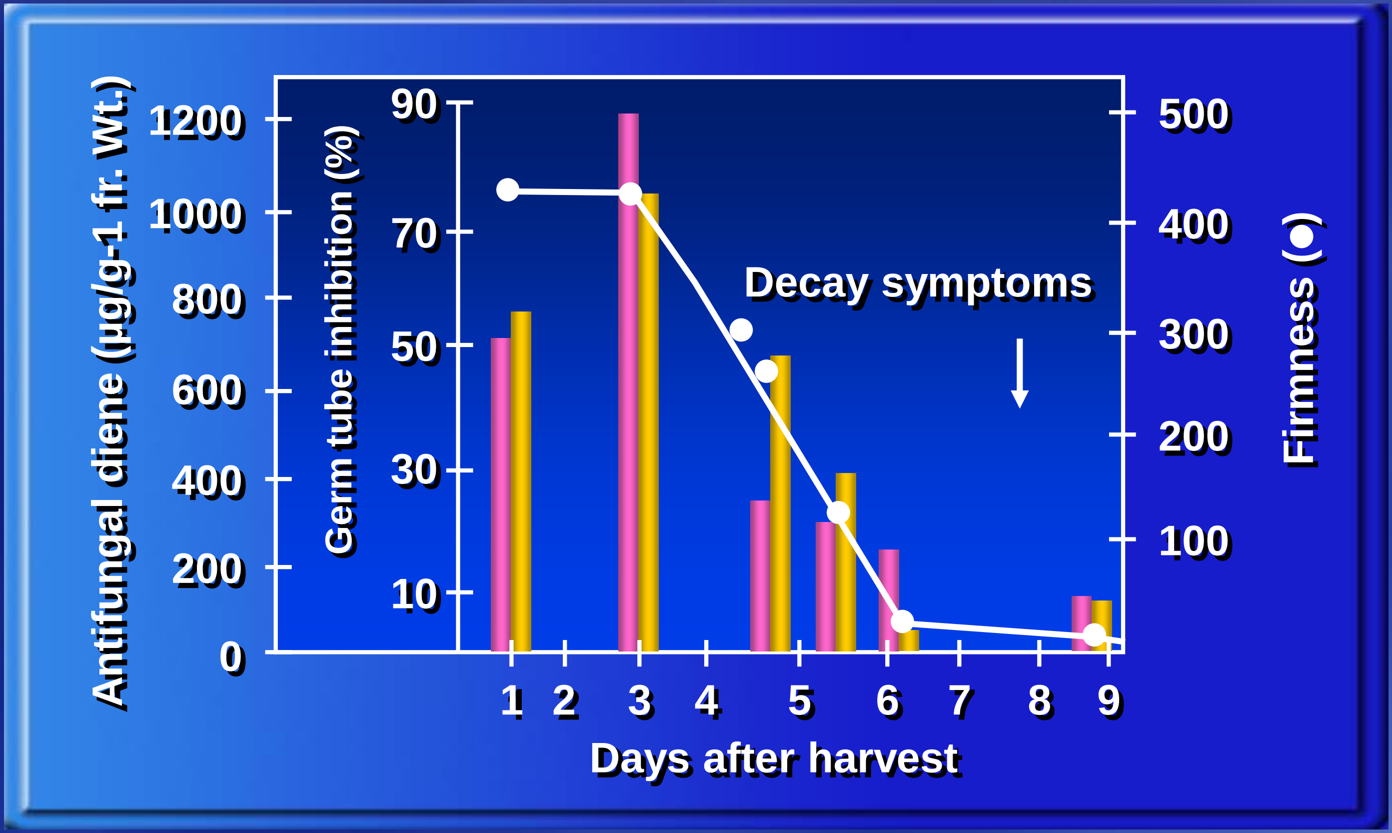
MONO-ENE

1-acetoxy-2,4-dihydroxy-n-heptadeca-16-ene (1986)

Decline of antifungal compounds in the peel of avocado fruit



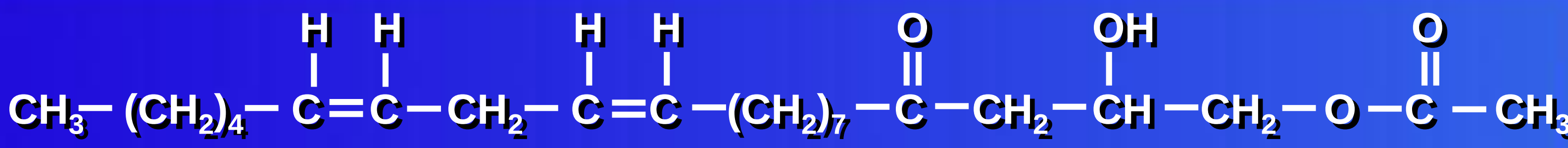
The relation between fruit ripening and antifungal activity of the avocado peel extract

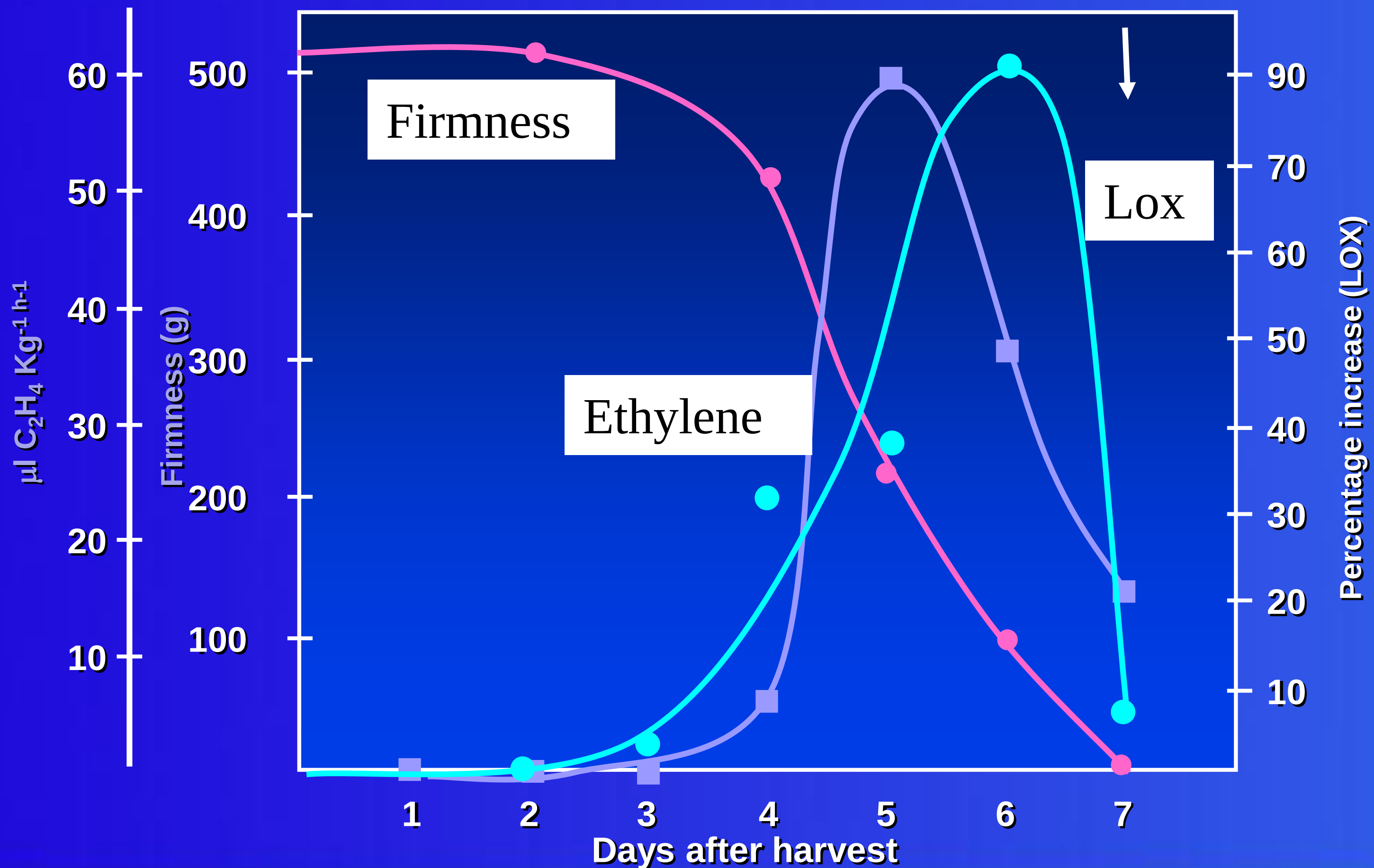


Fruit firmness (●), antifungal activity ( ), and concentration of the antifungal diene ( ) in crude extracts from peel of avocado cultivar Fuerte at different stages after harvest. The arrow denotes the first visible decay symptoms

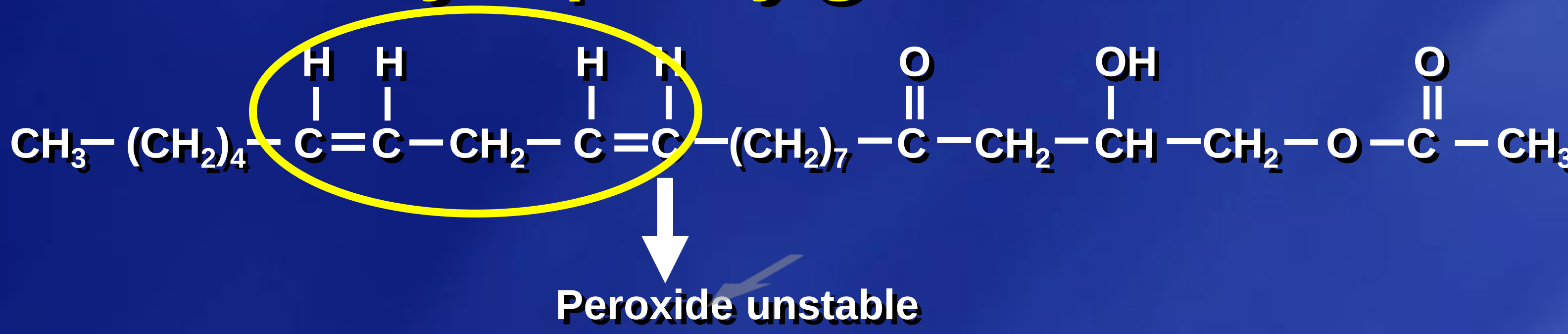
Why is the compound declining?

The involvement of lipoxygenase in the metabolism of the diene

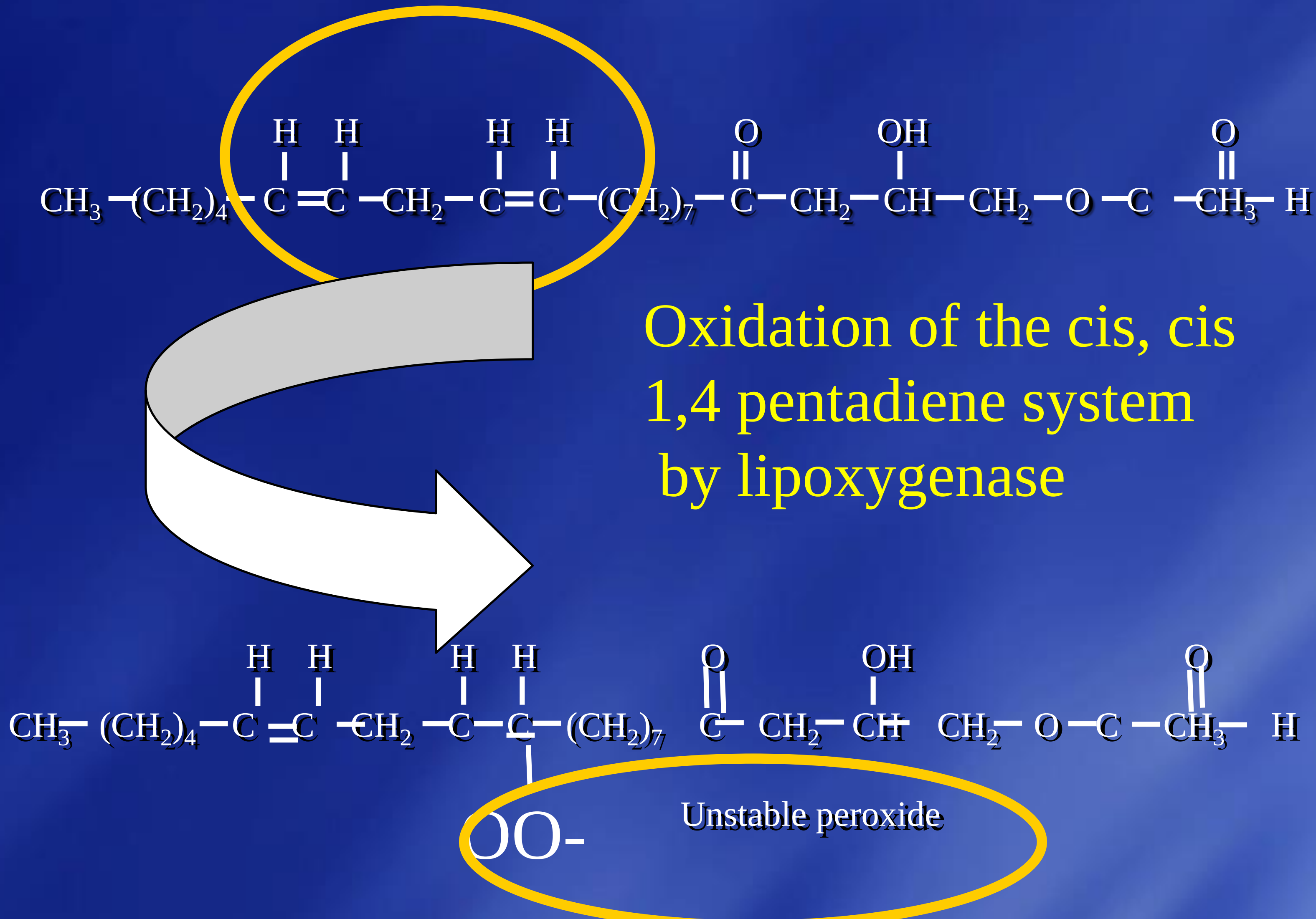




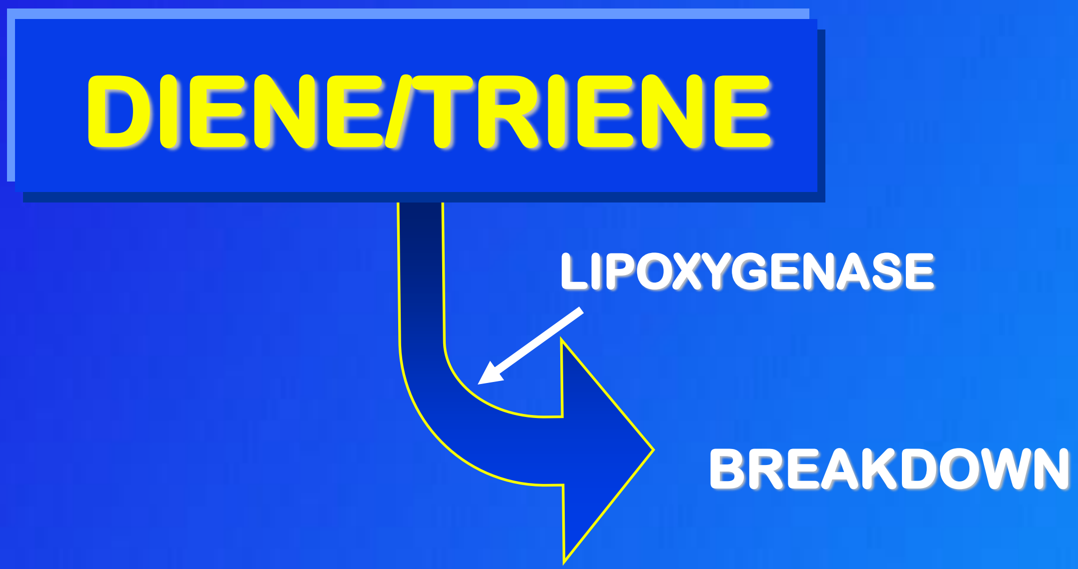
The mechanism involved in the metabolism of the diene by lipoxygenase



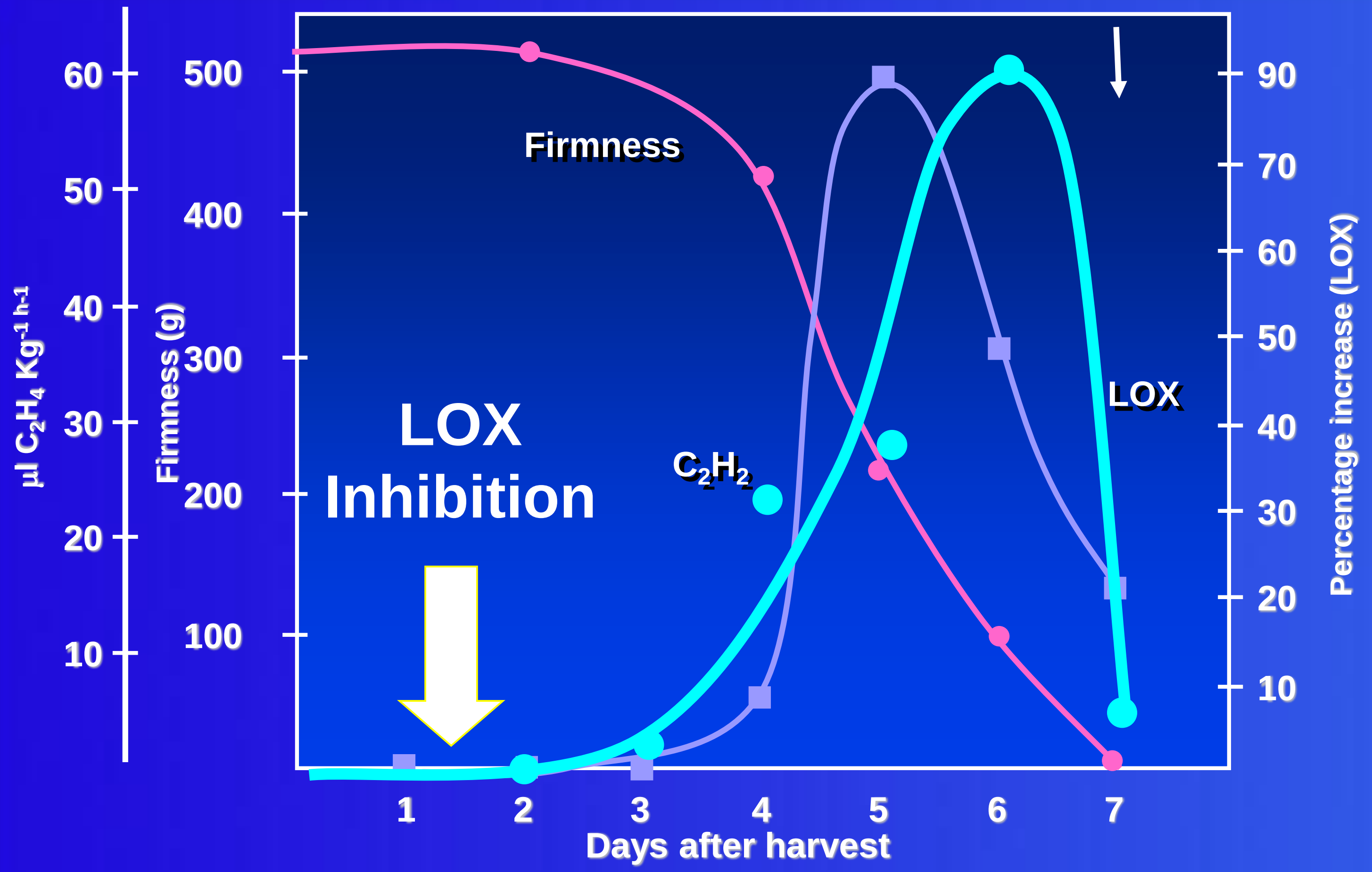
Diene metabolism



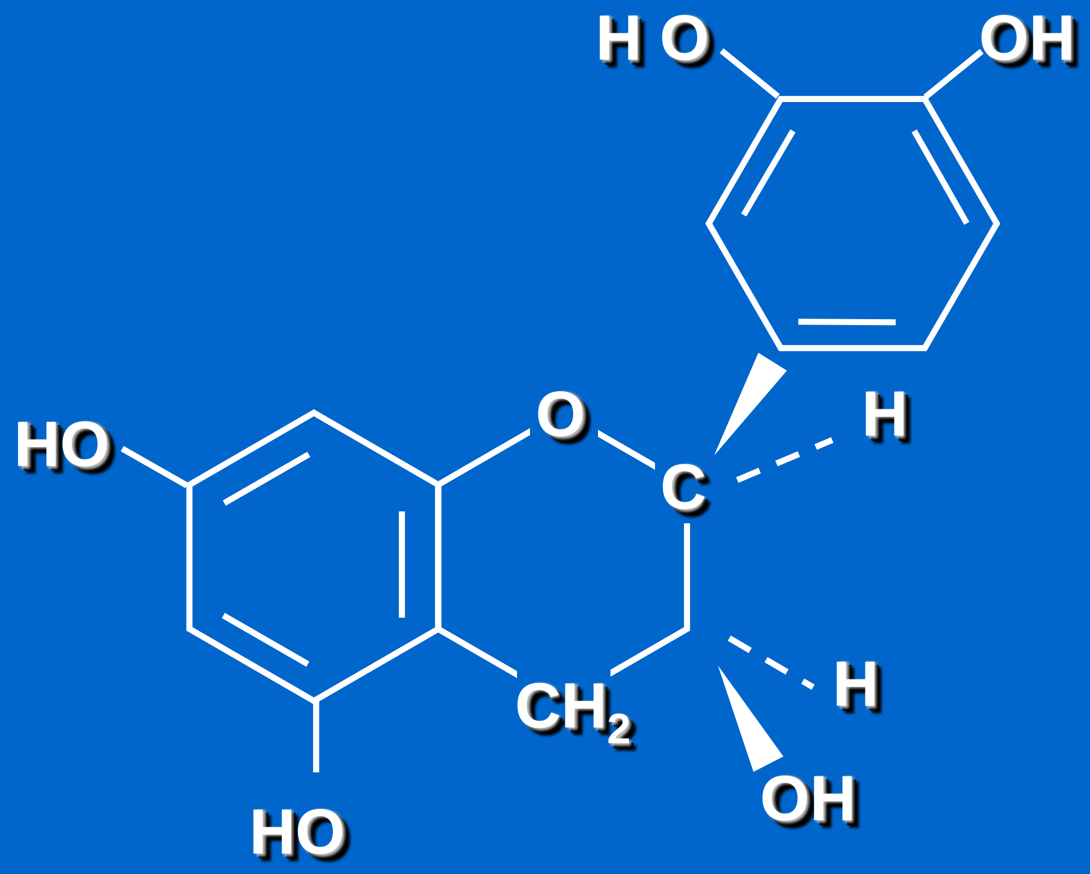
Oxidation of the cis, cis 1,4 pentadiene system by lipoxygenase


DIENE/TRIENE
LIPOXYGENASE
BREAKDOWN

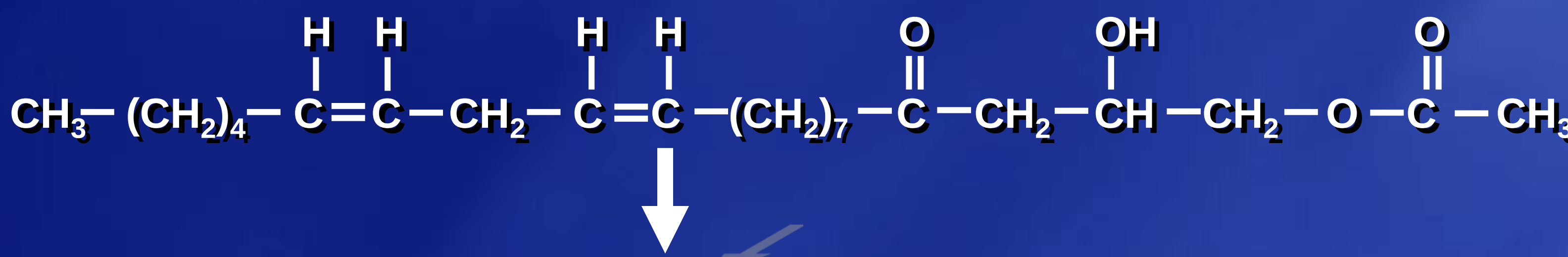
Lipoxygenase involvement in the breakdown of the Diene



Epicatechin

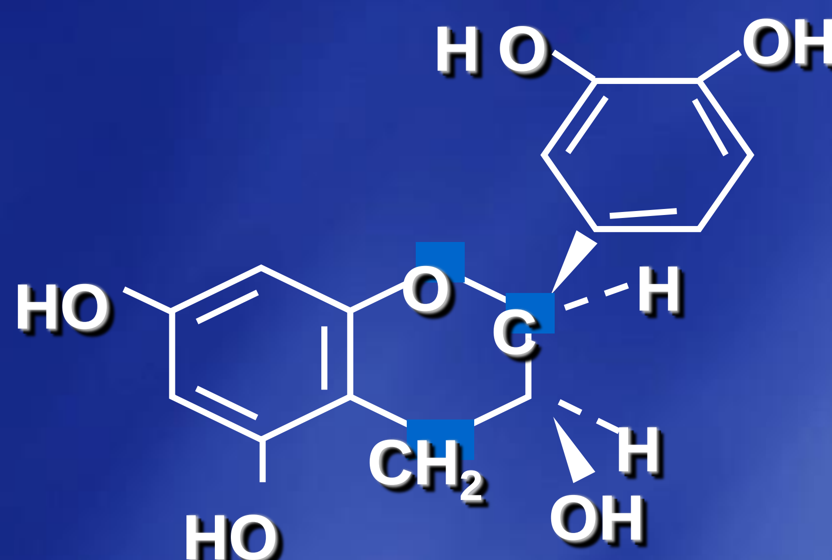


The involvement of epicatechin in the catabolic inhibition of lipoxygenase and the metabolism of the diene

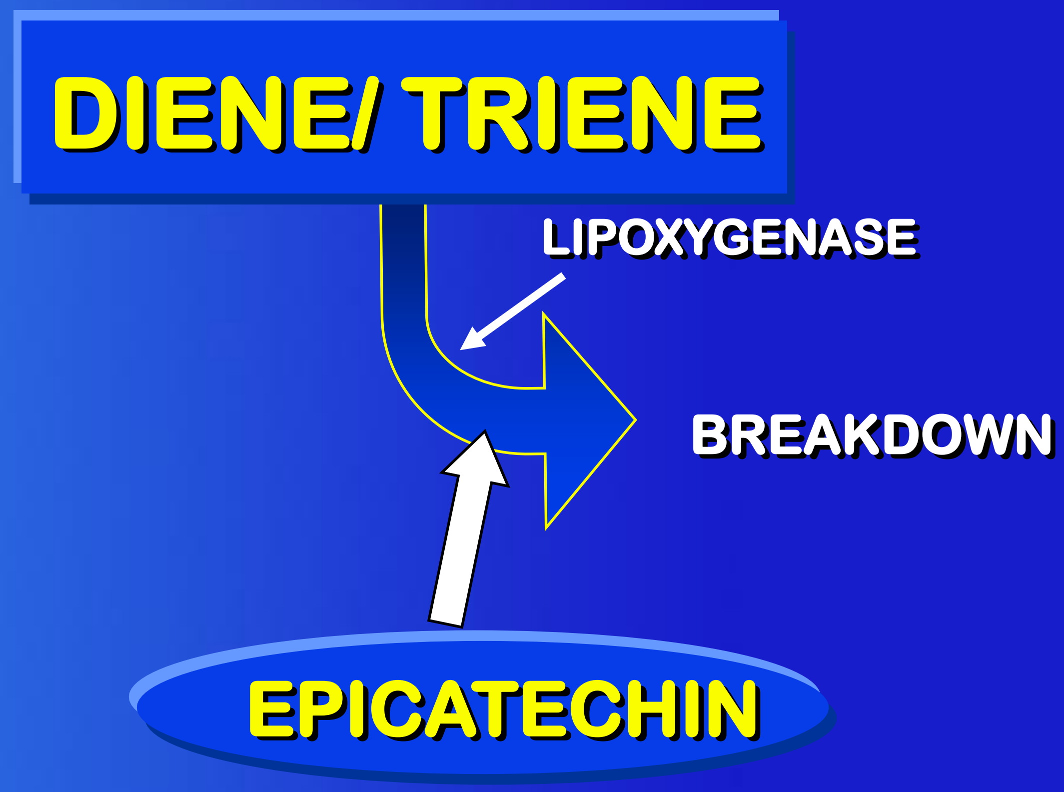


Peroxide unstable

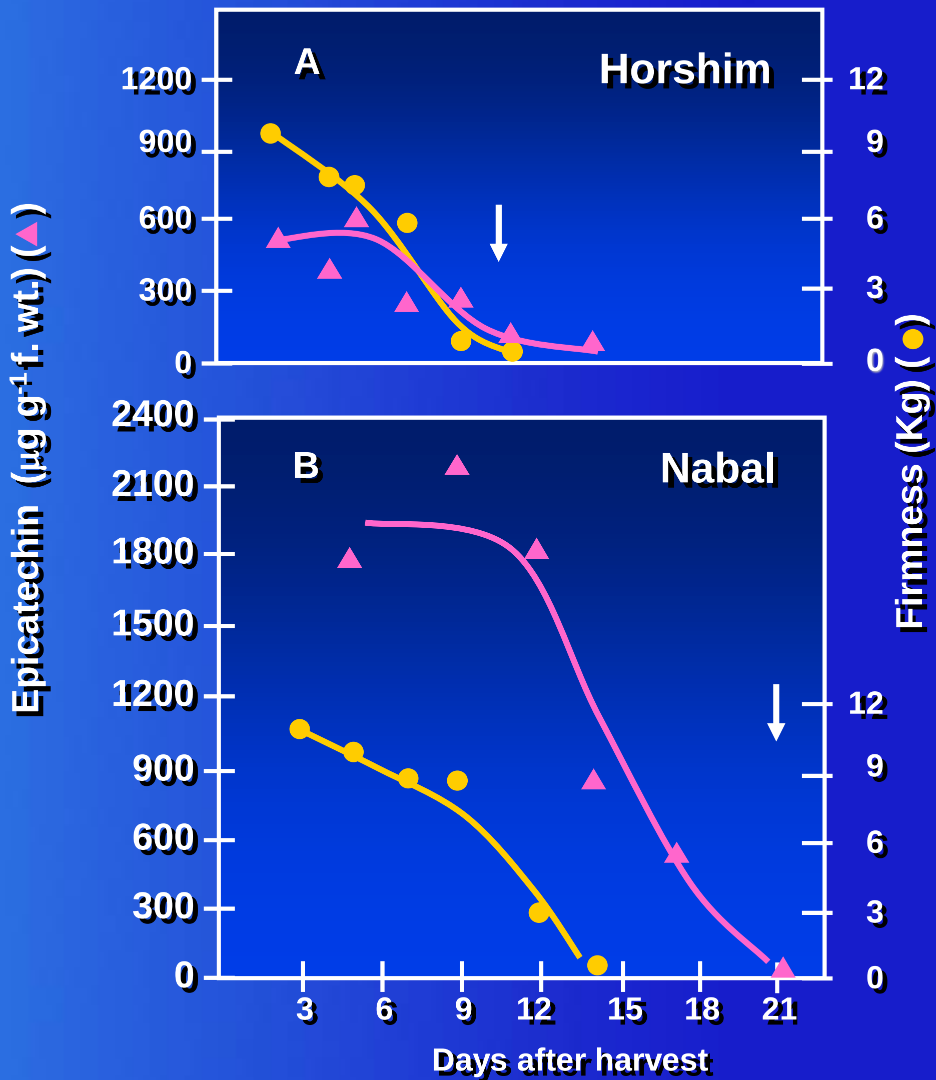
Instead



Oxidation of the antixodiant


DIENE/ TRIENE
LIPOXYGENASE
BREAKDOWN
EPICATECHIN

The relation between decrease in concentration of epicatechin and cultivar susceptibility



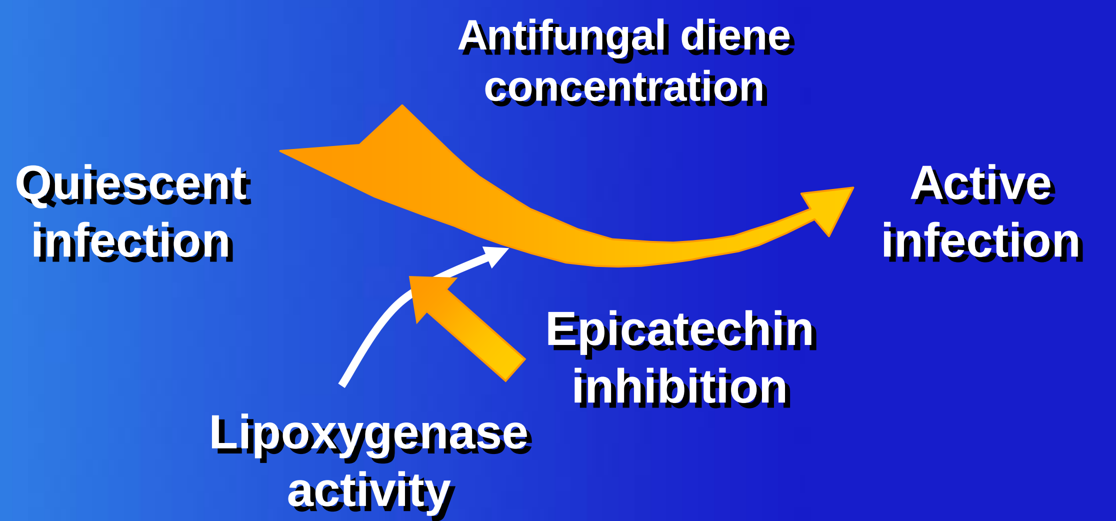
SUSCEPTIBLE CULTIVAR

RESISTANT CULTIVAR

Concentrations of epicatechin, fruit ripening and disease development

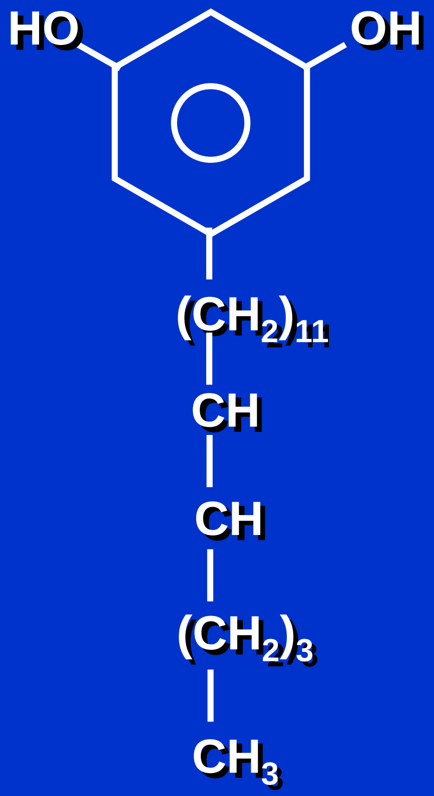
Cultivar	**Epicatechin at softening ($\mu g.g^{-1}$f.wt)**	Days to softening	**Days to Anthracnose**
Fuerte	60	11	11
Rincon	70	10	10
Vurtz	100	15	15
Benik	110	16	16
Nabal	822	14	24
Hass	1500	16	24

The relation between decrease in concentration of the antifungal compounds and activation of quieiscent infections

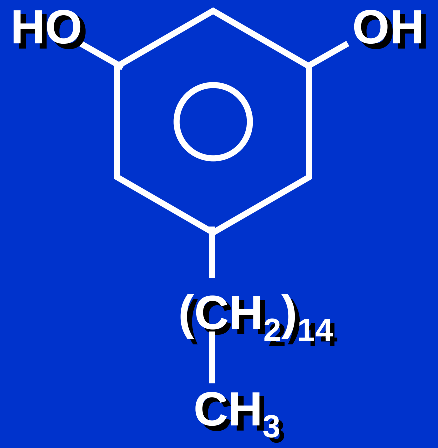


Relationship between relative diene concentration (as thickness of arrow), lipoxygenase activity, and epicatechin levels during avocado fruit ripening and susceptibility to anthracnose decay.

Antifungal compounds of unripe mango fruits

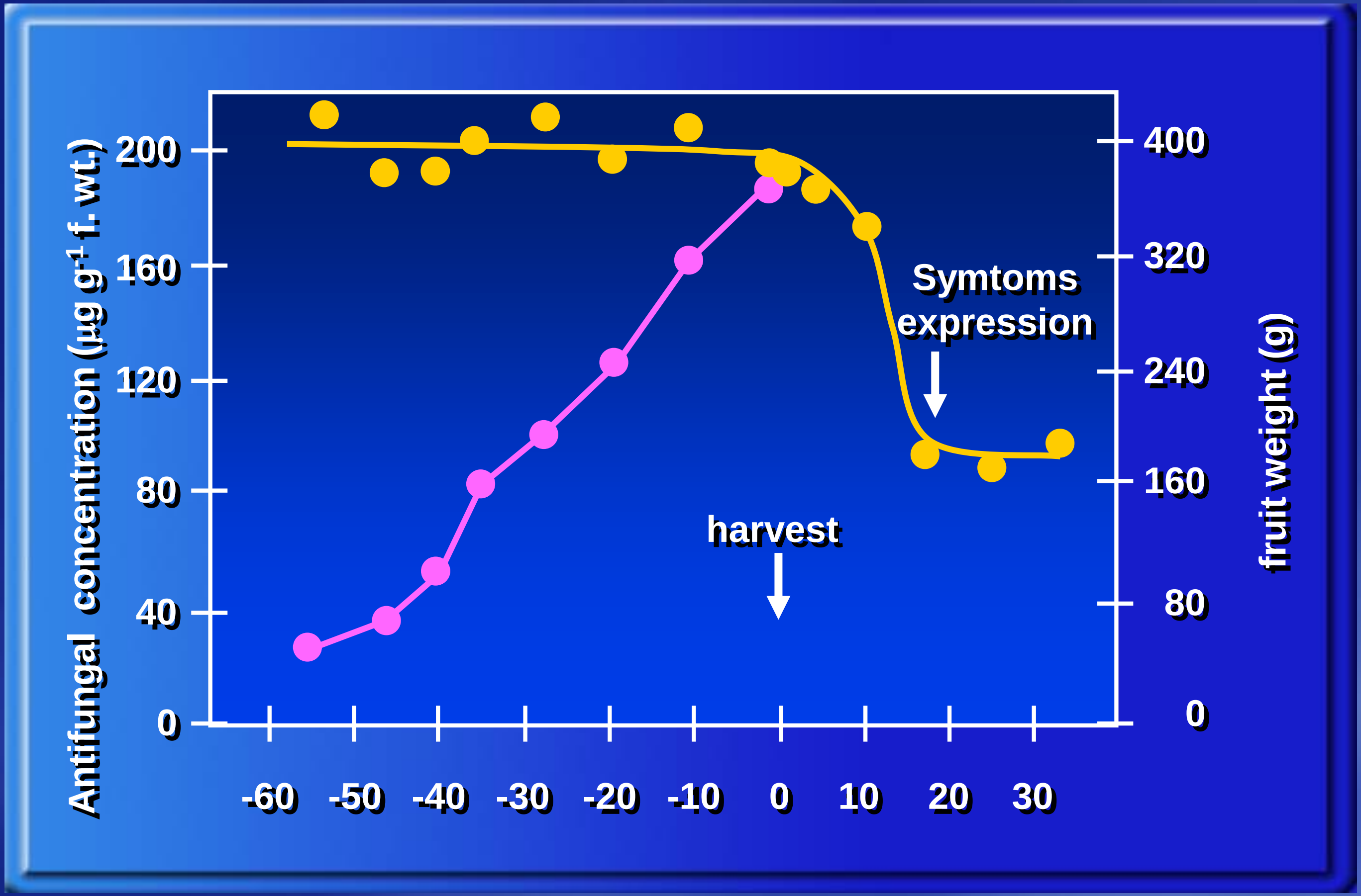


Resorcinol-5-(12-heptadeceny)



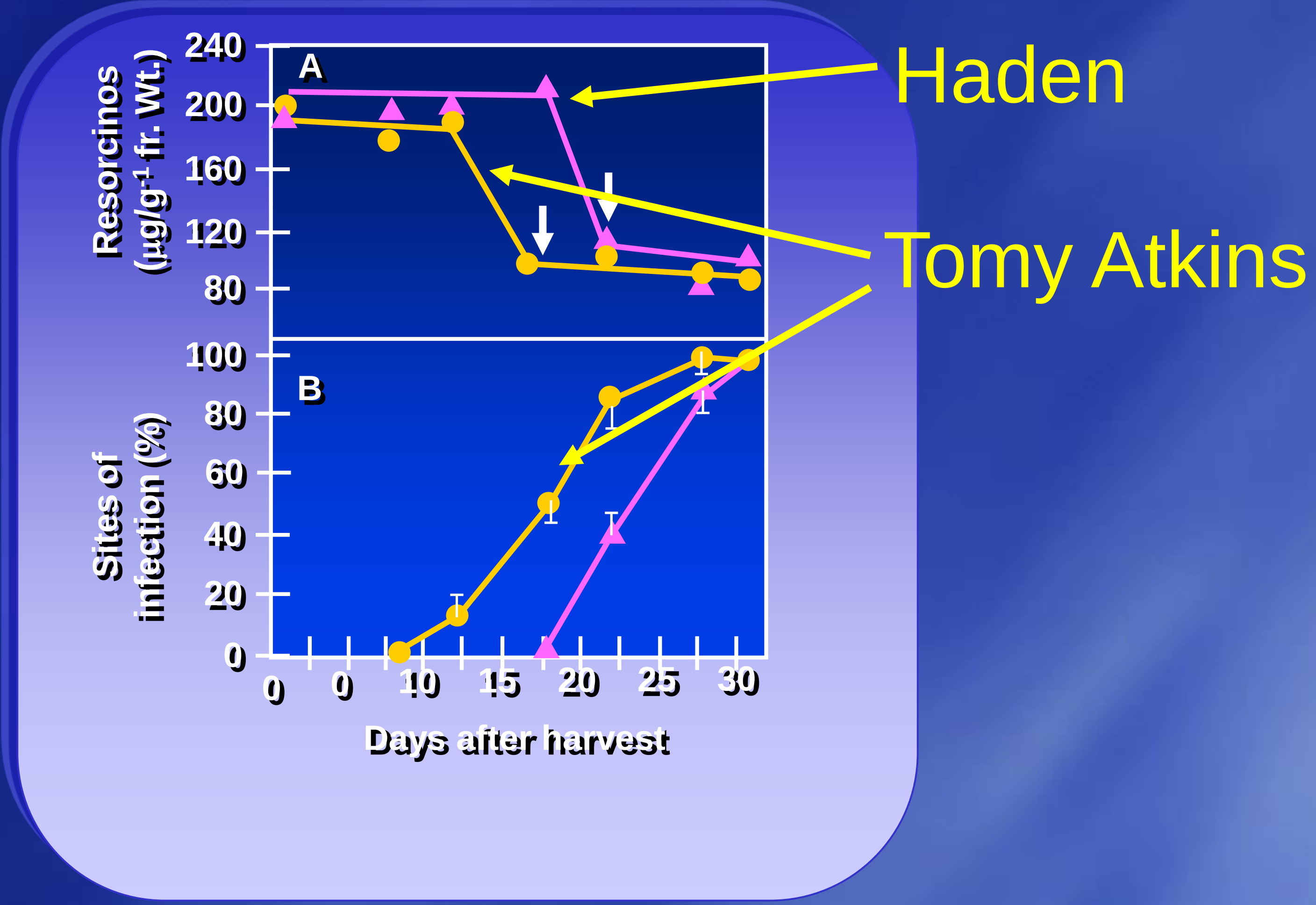
Resorcinol-5-(pentadecyl)

Differential level of antifungal compounds in mango fruit and their effect on symptom development

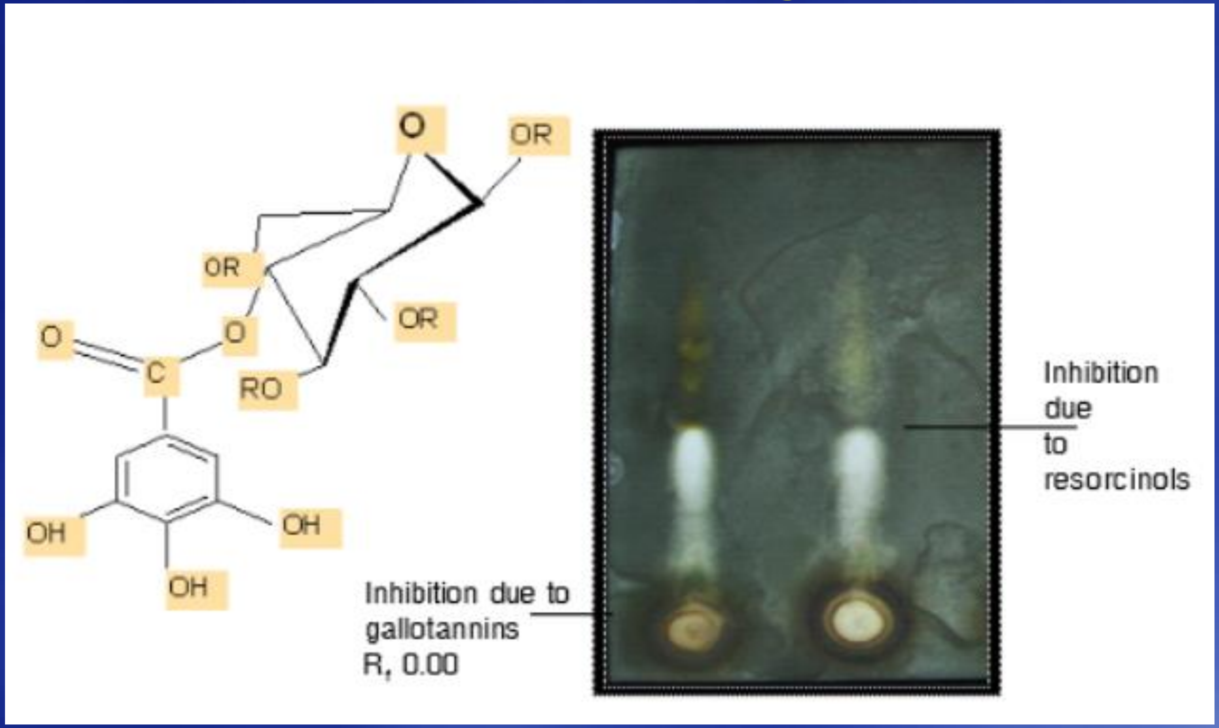


Droby et al. 1980

Differential level of antifungal compounds in mango fruit and their effect on Alternaria symptoms



Structure of Gallo tannins and thin layer chromatography bioassay of dicholormethane:methanol extracts of unripe mango

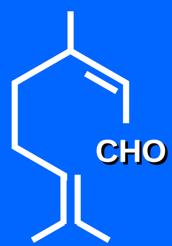


During mango ripening (break stage) the antifungal activity had declined by about 20% from what it was at harvest.

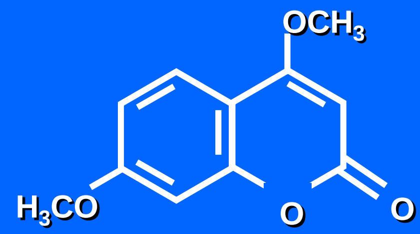
Citrus antifungal compound

geronial

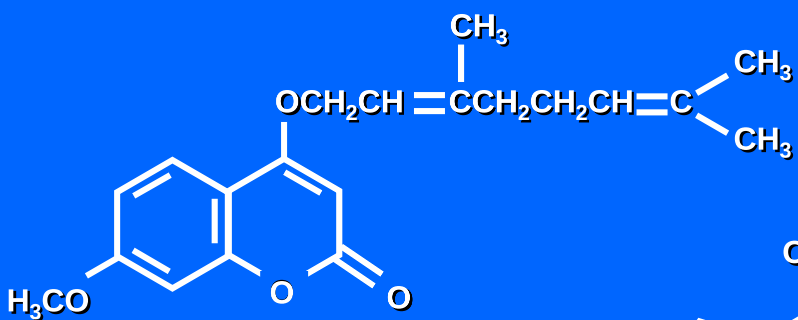
Citral



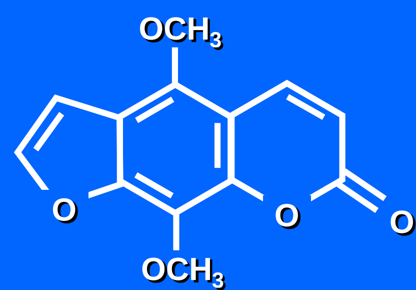
neral



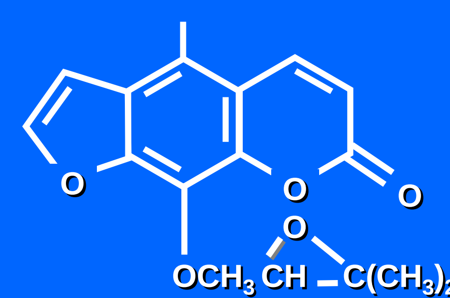
Limettin



5-geranoxy, 7-methoxycoumarin



Isopimpinellin



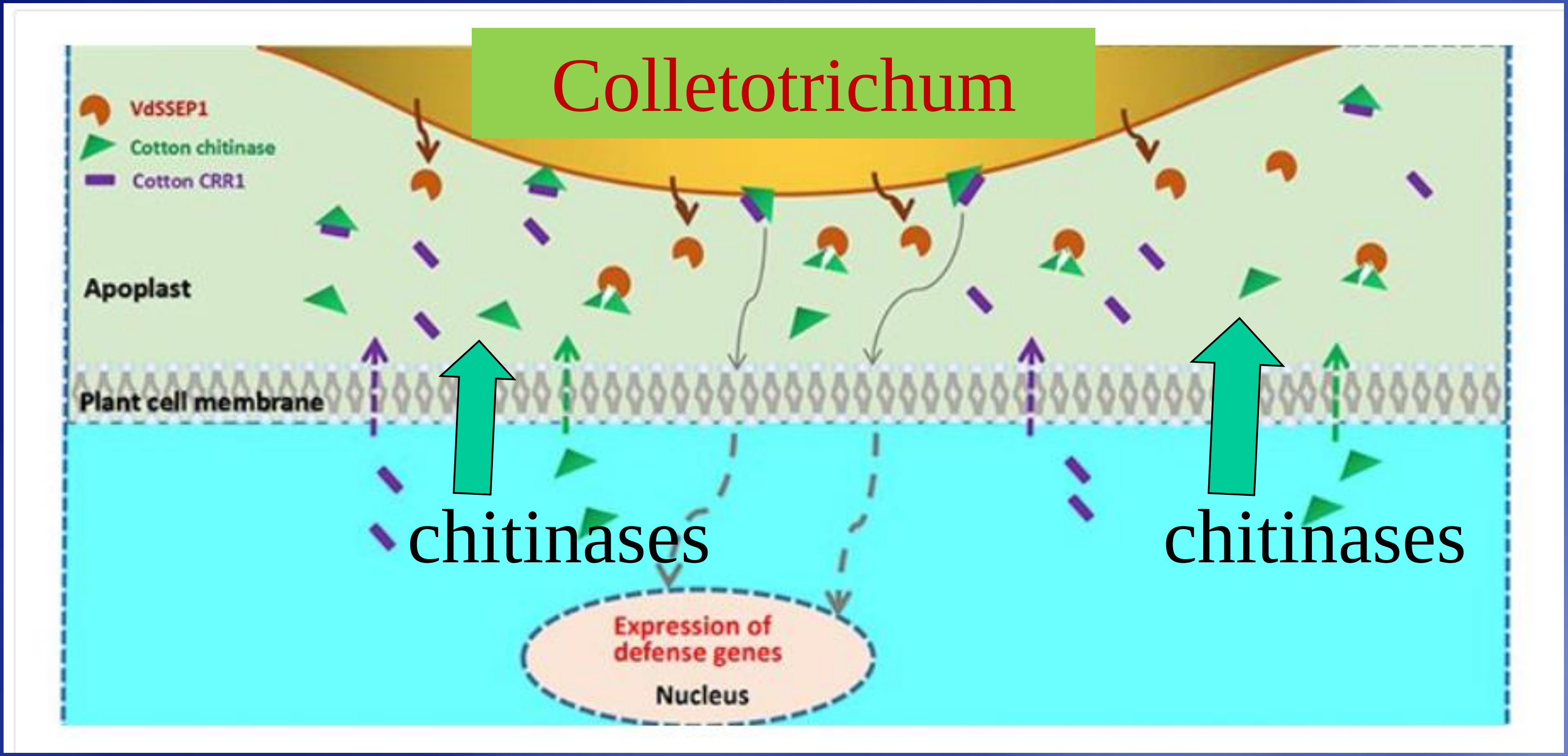
Oxyimperatarin

Preformed inhibition

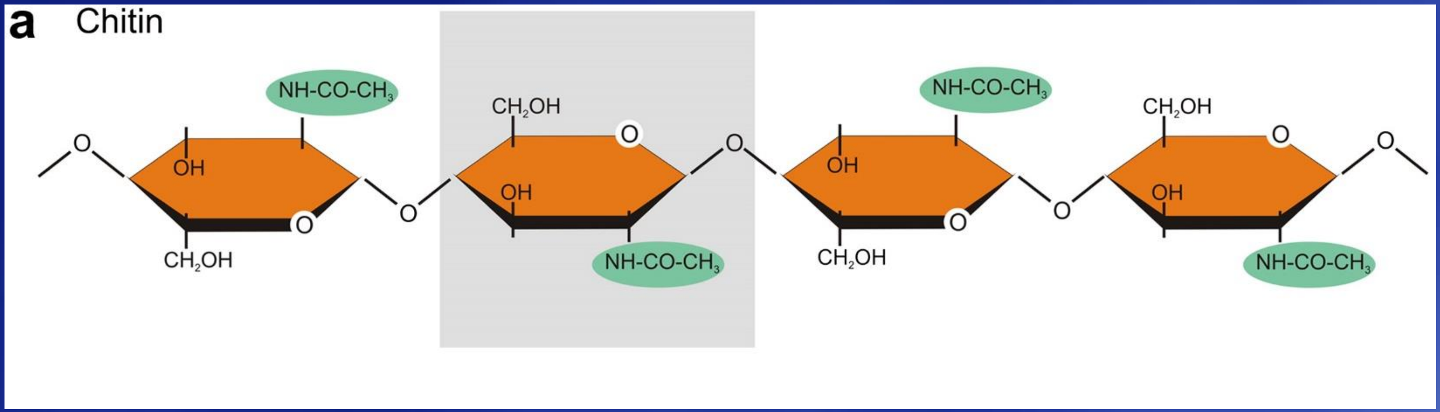
- We described “Preformed natural antifungal compounds that inhibit fungal growth”
- Preformed natural enzymes that inhibit fungal growth

Mango Latex containing Chitinases

Host Chitinases affecting fungal attack



Fungal chitin
N-acetyl glucose ammine



Antifungal enzymes of mango latex on spore germination

- **Chitinases were present in the aqueous phase of mango latex which hydrolyzes cell wall of *Colletotrichum* spp.(Karunanayake et al., 2011)**

- **The level of chitinase activity in the aqueous phase varied with the mango cultivar. The chitinase activity was higher in the cultivar resistant to anthracnose**

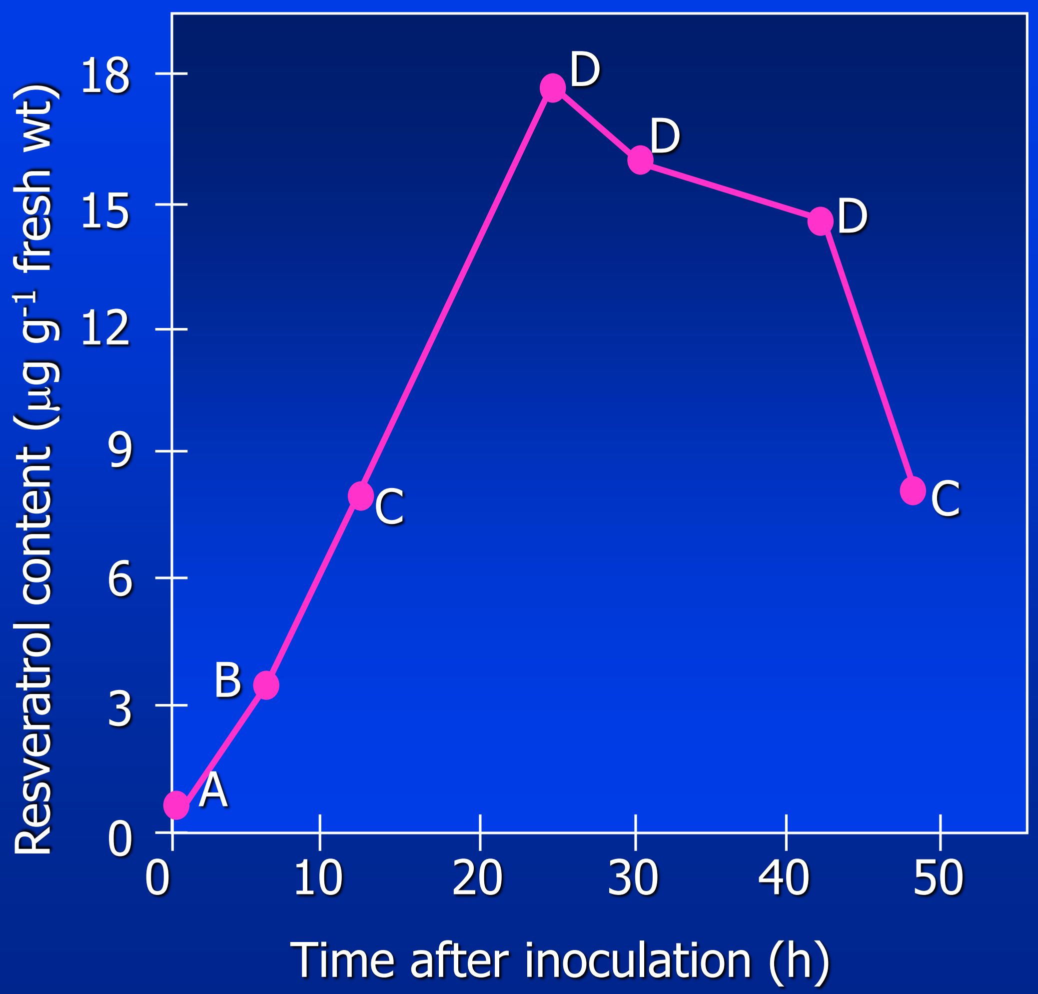
Mechanism contributing to activation of quiescence and fungal colonization

1. The need of host nutritional factors for fungal initial fungal colonization
2. The decline of host preformed antifungal compounds during fruit ripening.
3. Occurrence of host inducible limiting compounds and that decline during fruit ripening.
4. Activation of fungal pathogenicity factors during ripening fruits.

Inducible compounds that decline during fruit ripening

These host inducible response is only observed in unripe fruits but not in ripe ones!!!

Induction of resveratrol



Phytoalexin elicitation in infected berries

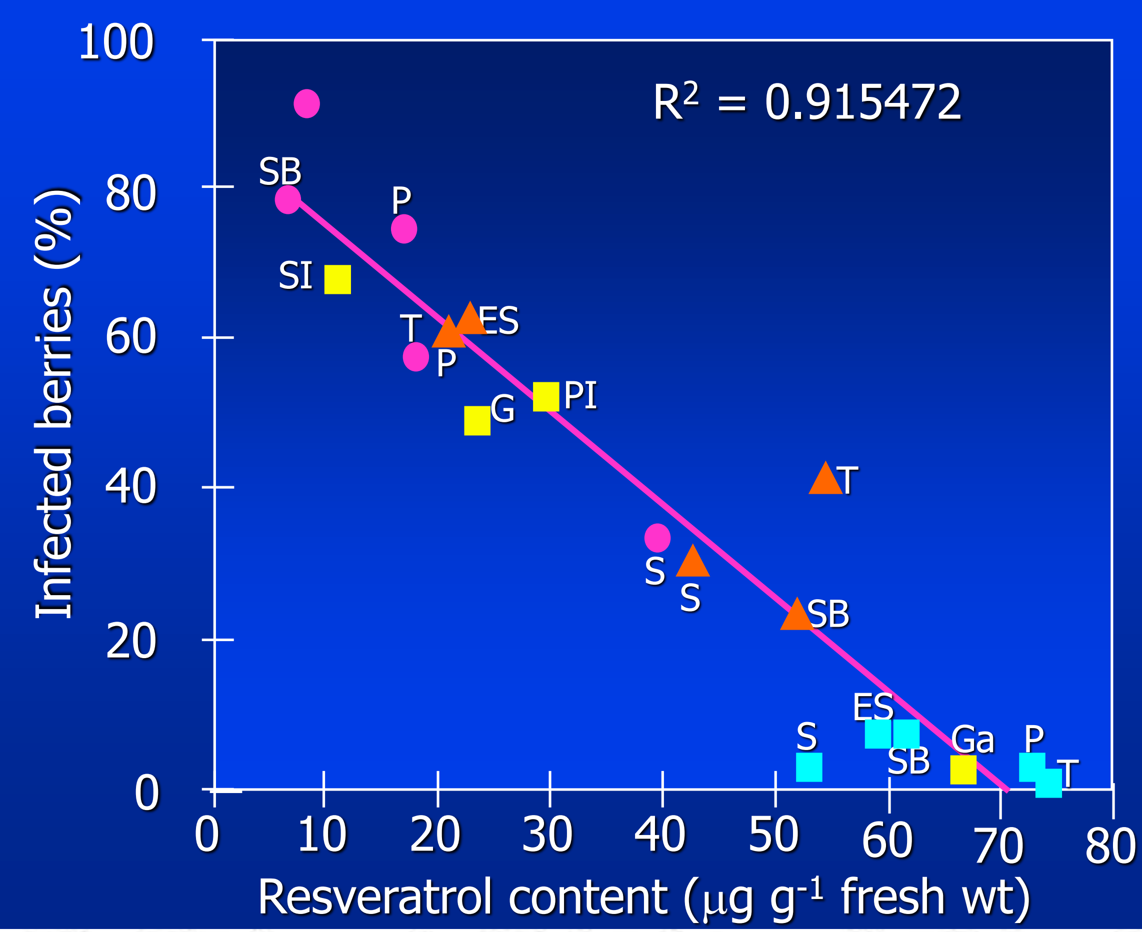
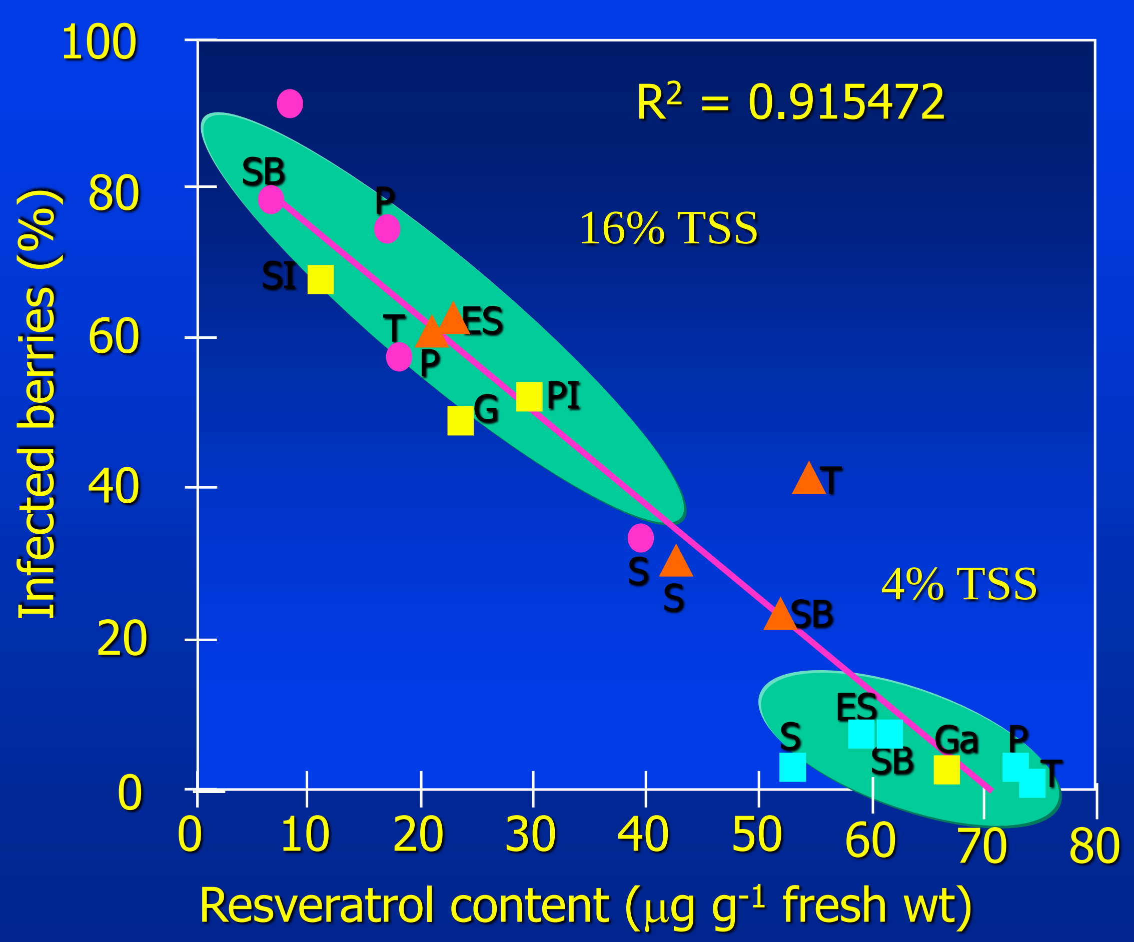


FIG. 4. Correlation between the elicitation of resveratrol by 10 min u.v.-C irradiation and the extent of *Rhizopus* decay in inoculated grape berries of cultivars ES (Early Superior), G (Gamay); Ga (Gamaret); P (Perlette); Pi (Pinot); S (Superior); SB (Spring Blush); Sh (Shasla) and T (Thompson Seedless) harvested at different stages of development (based on % SSC): (■) 4%; (▲) 6%; and (○) 16% SSC.

Phytoalexin elicitation in infected berries



Inducible factors modulating fungal colonization

ROS production

during the interaction of the pathogen and the fruit

Was reported:

Production of reactive oxygen species (ROS) has been recognized as one of the earliest induced defense responses in plants

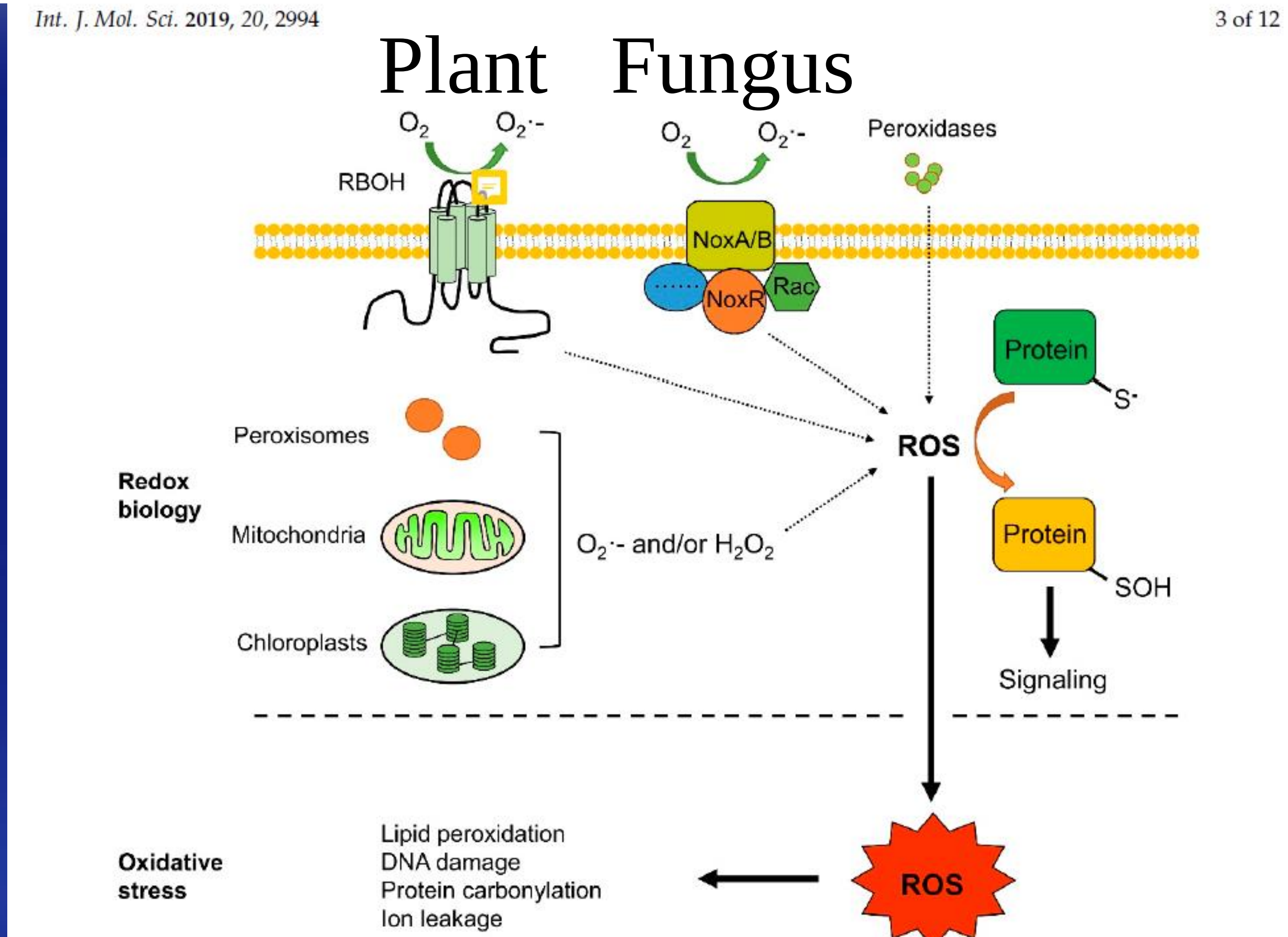
Three degree of ROS interactions

1. <u>In the host</u> , pathogens –ROS interaction generated by the host resulting in a direct killing of the pathogen

2. <u>In the host</u>, host cell death caused by ROS may lead to cellular necrosis in the hosts, close to quiescent pathogens (hemibiotrophic or necrotrophic) from where it acquire nutrients, switching into the necrotrophic mode

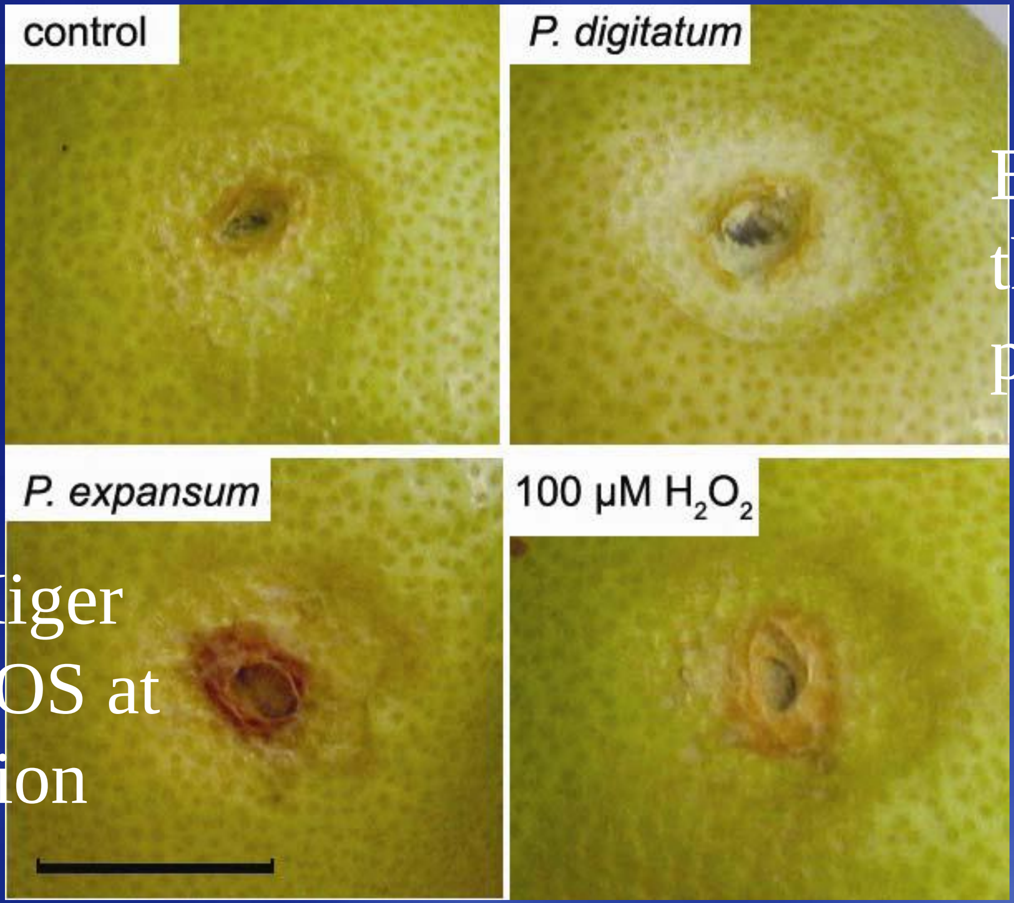
3. <u>For pathogens</u>, ROS play an important role in their infection processes.

Generation sites of reactive oxygen species (ROS) and redox biology.



ROS are produced by respiratory burst oxidase homologs (RBOHs), mitochondria, chloroplasts, peroxisomes, and cell wall-resident peroxidases (PER). Subsequent H2O2 accumulation may oxidize cysteine residues in proteins, aect their redox states and functions, and regulate related signaling pathways. Excessive ROS may lead to oxidative stress, which may cause lipid oxidation, DNA damage, protein carbonylation, and injuries to other cellular components.

Host response to the penetration hyphae of germinated spores, Macarisin et al., 2007



Enhanced ROS at the inoculation point

Non-Host pathogen. Higer enhanced ROS at the inoculation point

Detection of H_2O_2 in lemon fruits by diaminobenzidine (DAB) staining. The appearance of brown DAB polymers indicates accumulation H2O2 in response to wounding (control),

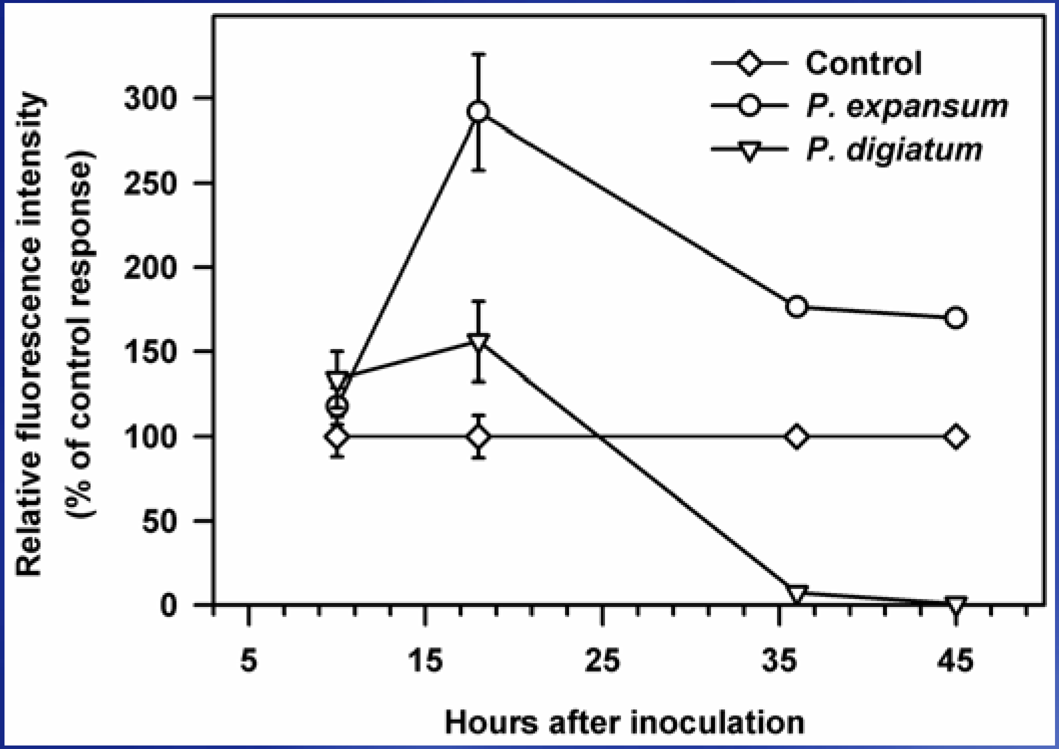
Host response to Penicillium

TABLE 1. Time-course production of H_2O_2 in lemon peel in response to inoculation with *Penicillium digitatum* and *P. expansum*

Treatment	Time after inoculation (h) 18	42	66
P. digitatum	4.8 ± 0.3	6.9 ± 0.9	3.7 ± 0.3
P. expansum	3.3 ± 0.2	311.1 ± 25.4	112.7 ± 8.6
Control	4.6 ± 0.3	4.9 ± 0.2	9.8 ± 0.6

Concentration of ROS is expressed as relative pixel intensity

Penicillium digitatum suppresses production of H_2O_2 during infection of citrus fruit



H_2O_2 was measured by dichlorodihydrofluorescein-fluorescence upon inoculation with *Penicillium digitatum* or *P. expansum*, compared with uninoculated controls.

ROS Accumulation in Penicillium

Is regulated by activation of antioxidative processes by the host metabolism enabling fungal colonization

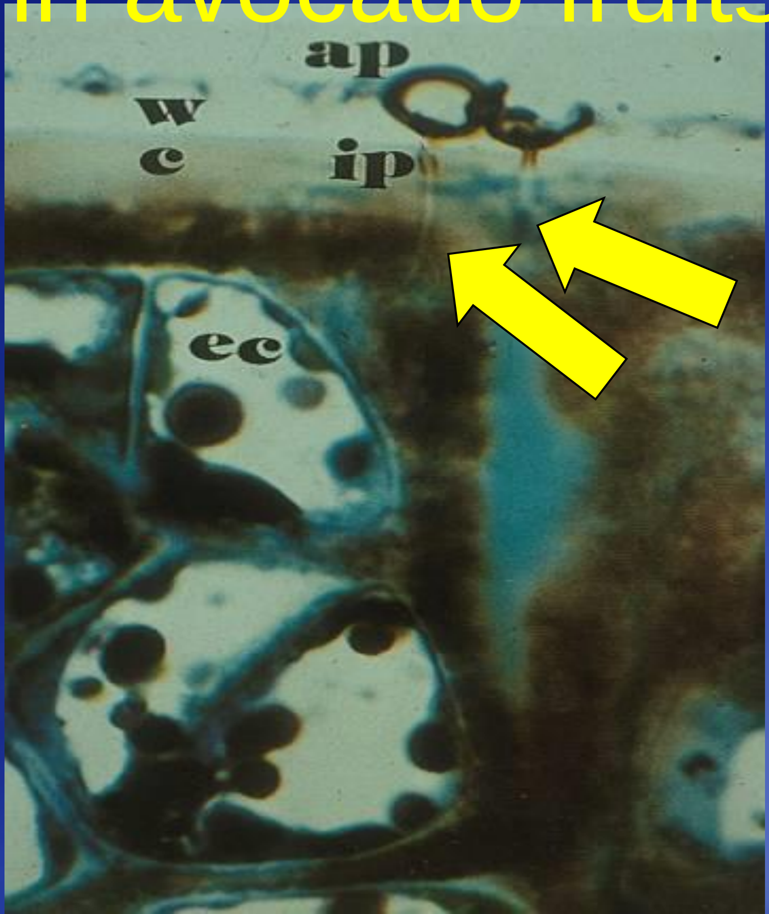
Three degree of ROS interactions

1. <u>In the host</u> , pathogens –ROS interaction generated by the host resulting in a direct killing of the pathogen

2. <u>In the host</u>, host cell death caused by ROS may lead to cellular necrosis in the hosts, close to quiescent pathogens (hemibiotrophic or necrotrophic) from where it acquire nutrients, switching into the necrotrophic mode

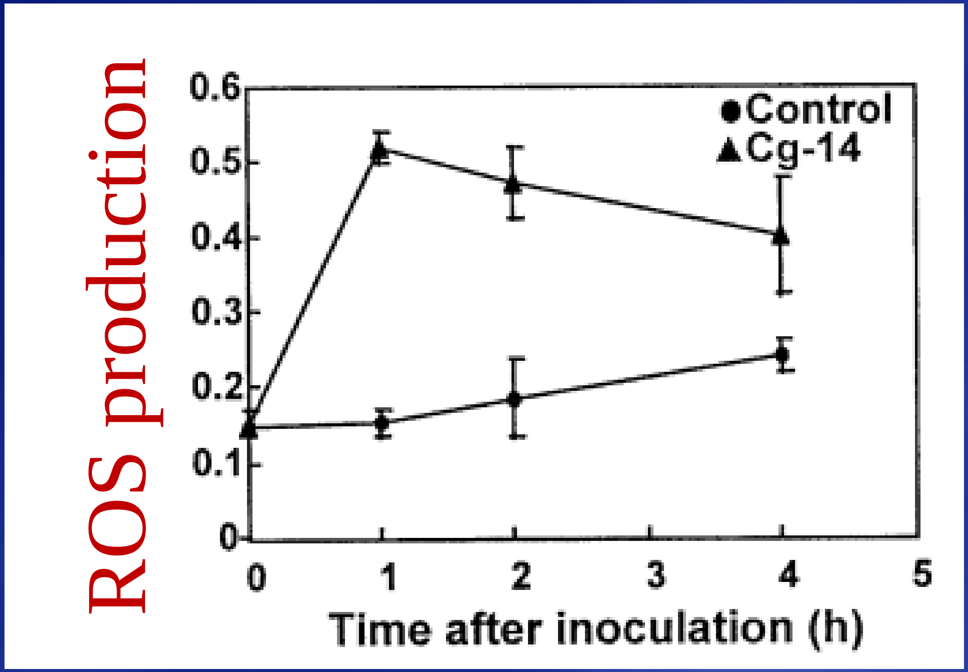
3. <u>For pathogens</u>, ROS play an important role in their infection processes.

Second example of host response:
Quiescence by Colletotrichum in avocado fruits

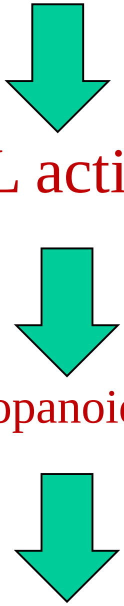


Second example of host response:

ROS production in plasma membranes from unripe avocado pericarp tissue inoculated with *Colletotrichum gloeosporioides spores*



Inoculation of unripe fruit Induced H_2O_2



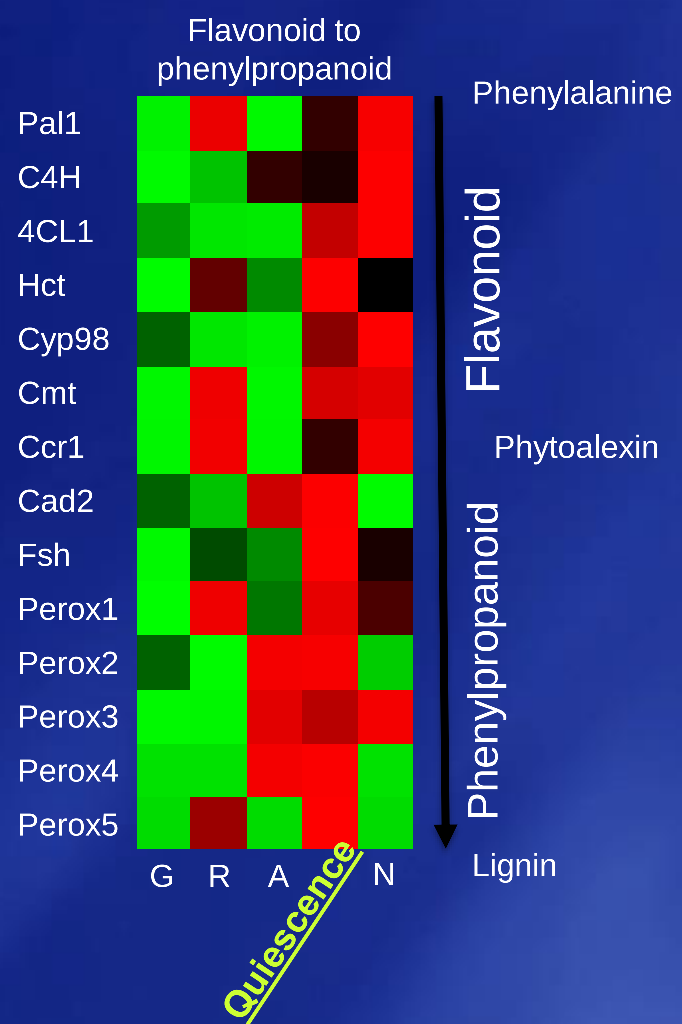
PAL activity

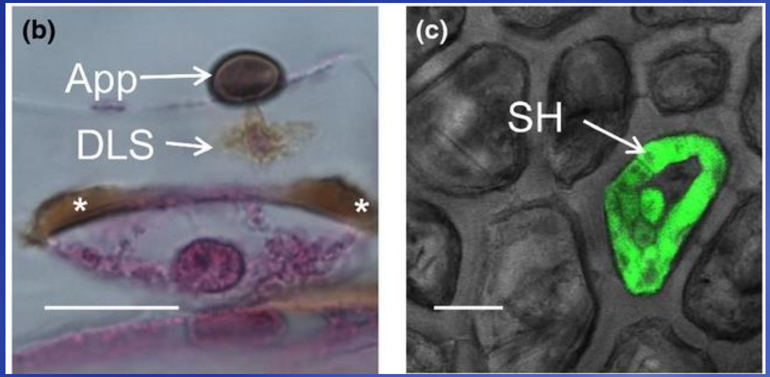
Phenyl propanoid pathway

Epicatechin

Beno Moualem et al., 2006

Mature-green tomato fruit prominent quiescent response to Colletotrichum





(Alkan et al., New Phytologist., 2015)

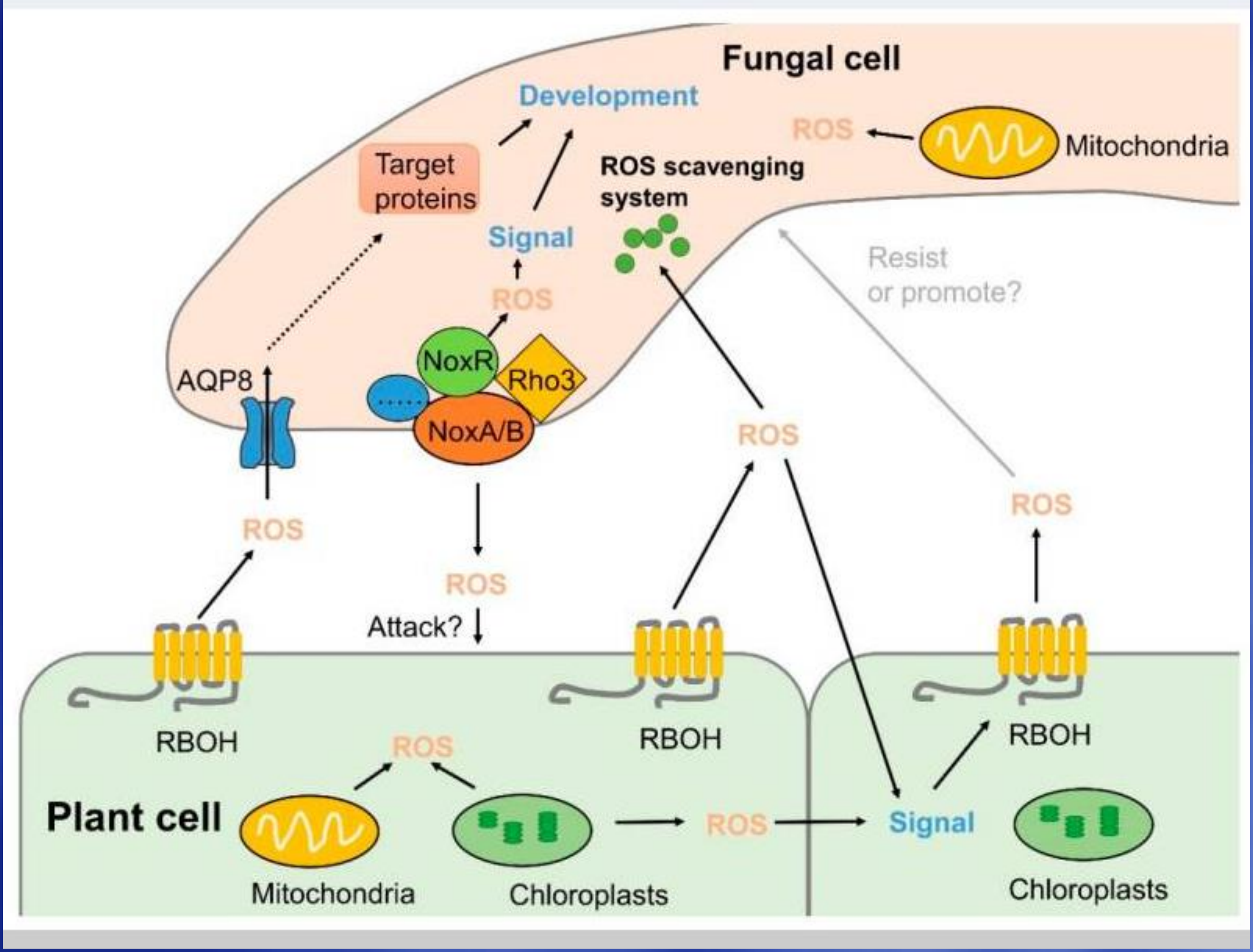
Three degree of ROS interactions

1. <u>In the host</u>, pathogens may encounter ROS generated by the host and as a result, they may be directly killed.

2. <u>In the host</u>, cell death caused by ROS may lead to cellular necrosis in the hosts, from which quiescent pathogens (hemibiotrophic or necrotrophic) acquire nutrients, switching into the necrotrophic mode

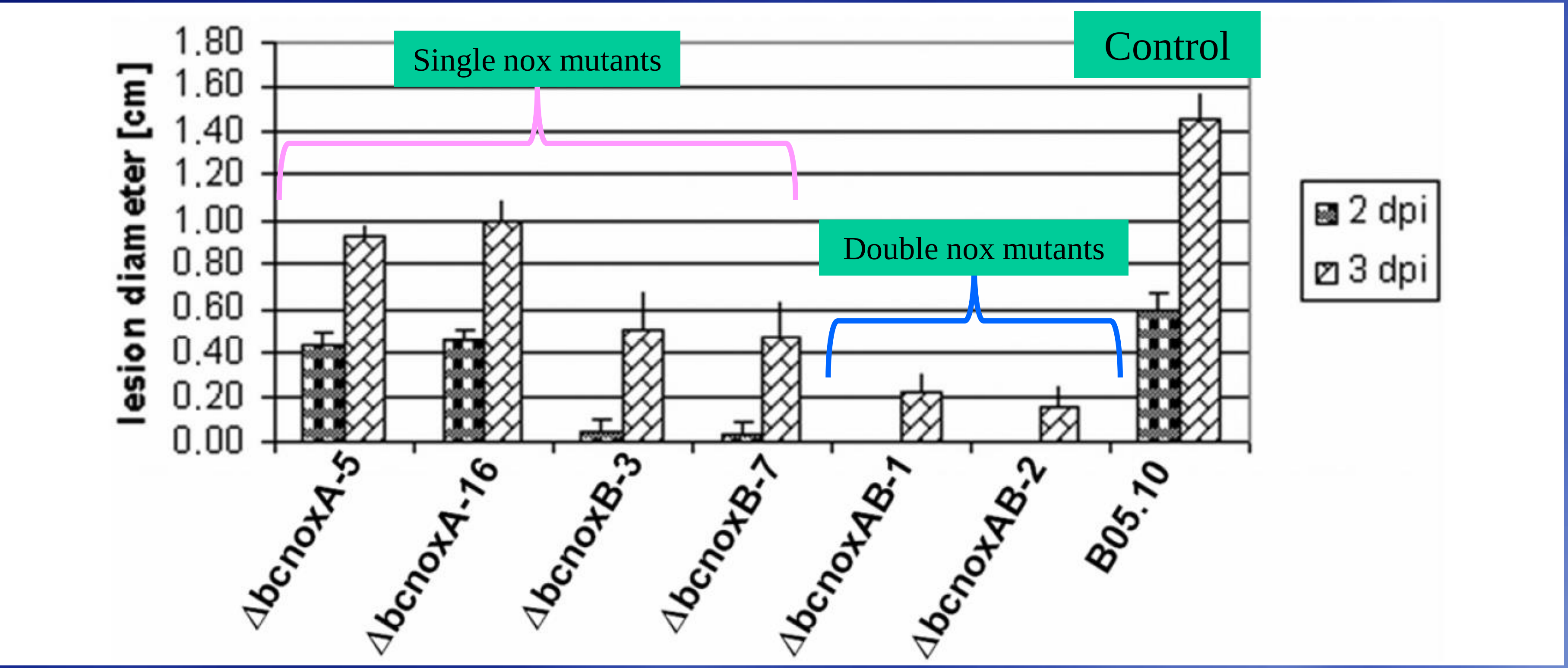
3. <u>For pathogens</u>, ROS play an important role in their infection processes.

Production, Signaling, and Scavenging Mechanisms of Reactive Oxygen Species in Fruit–Pathogen Interactions by Shiping Tian, International Journal of Molecular Sciences, 2019



Contribution of the ROS pathogen on pathogenicity

NADPH Oxidases are involved in differential pathogenicity in *Botrytis cinerea* in bean leaves



Segmüller and Tudzynski, MPMI 2009

Plant hormonal modification at initial stages of fruit colonization

Inducible factors modulating fungal colonization

Plant hormones production

during the interaction of the pathogen and the fruit

Hormone modulation of resistance in fruit pathogen interactions

Salicylic acid (SA) is signaling molecule in plant defense against biotrophic pathogen as *Colletotrichum*

while

JA is a signaling molecule in plant defense against necrotrophic pathogens as *Penicillium, Alternaria* and others

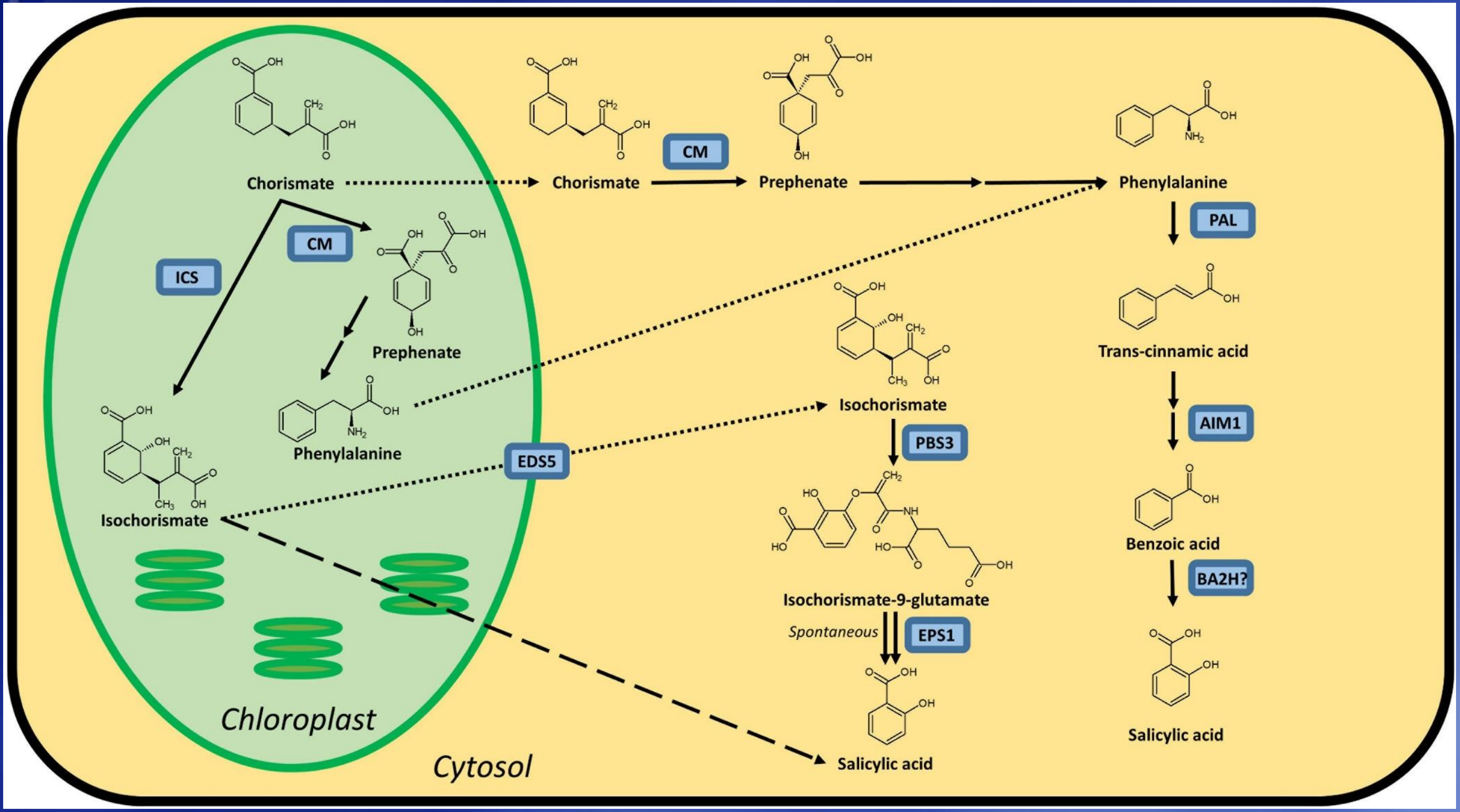
Salicylic acid

SA improved the resistance to *Alternaria alternata* and *P. expansum* by inducing the activity of anti-oxidant enzymes and pathogenesis-related proteins in sweet cheery.

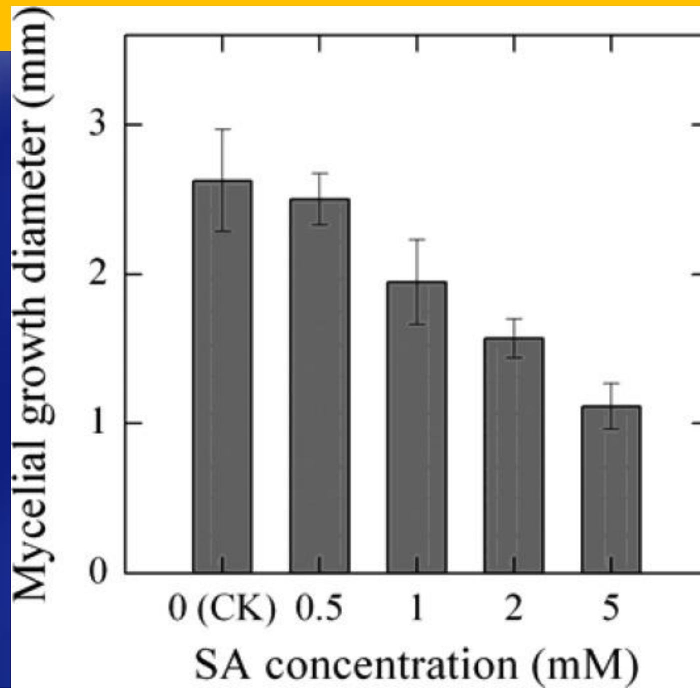
In citrus fruit SA reduced disease severity by inducing the accumulation of H_2O_2, primary metabolites, and flavones (phenols)

SA may also facilitate H_2O_2 accumulation during the oxidative burst induced by infection with virulent pathogens

Biosynthesis of SA

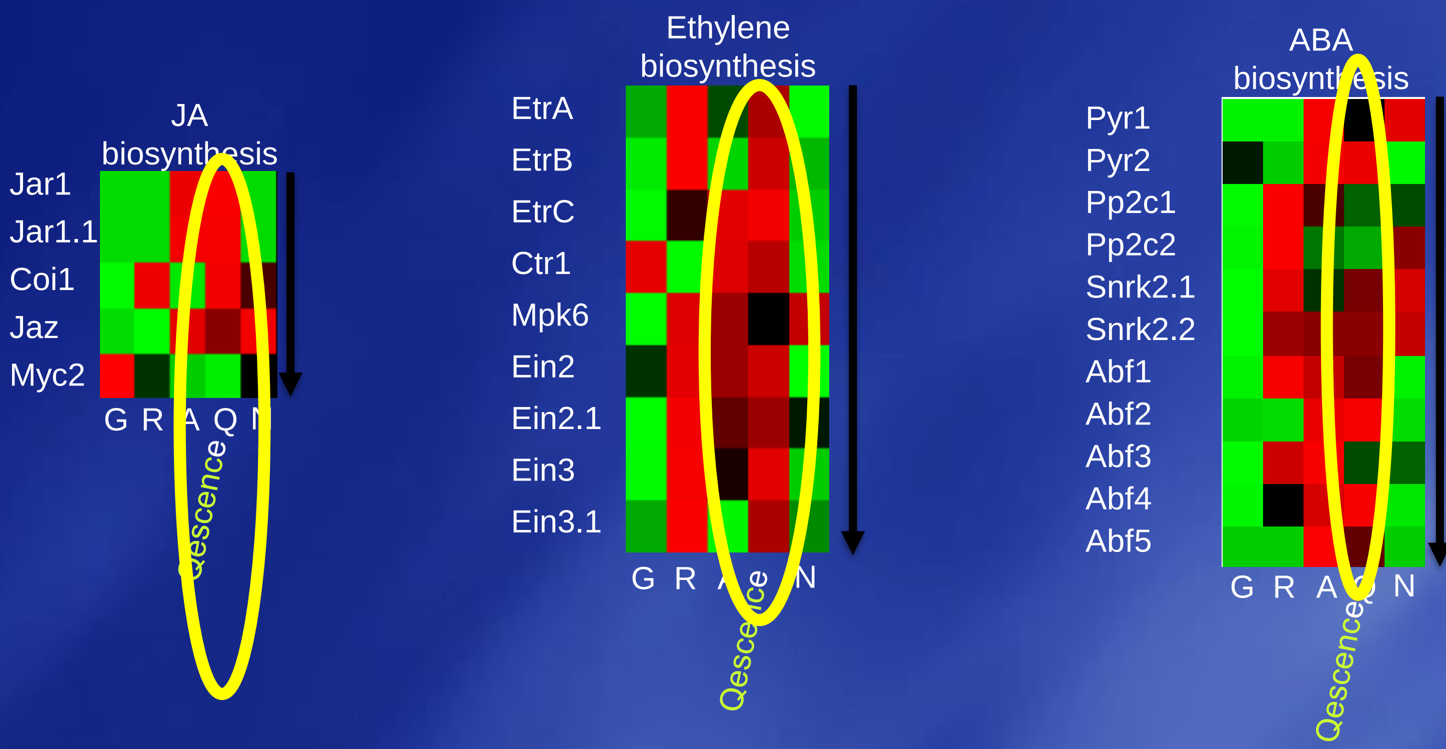


Effect of SA



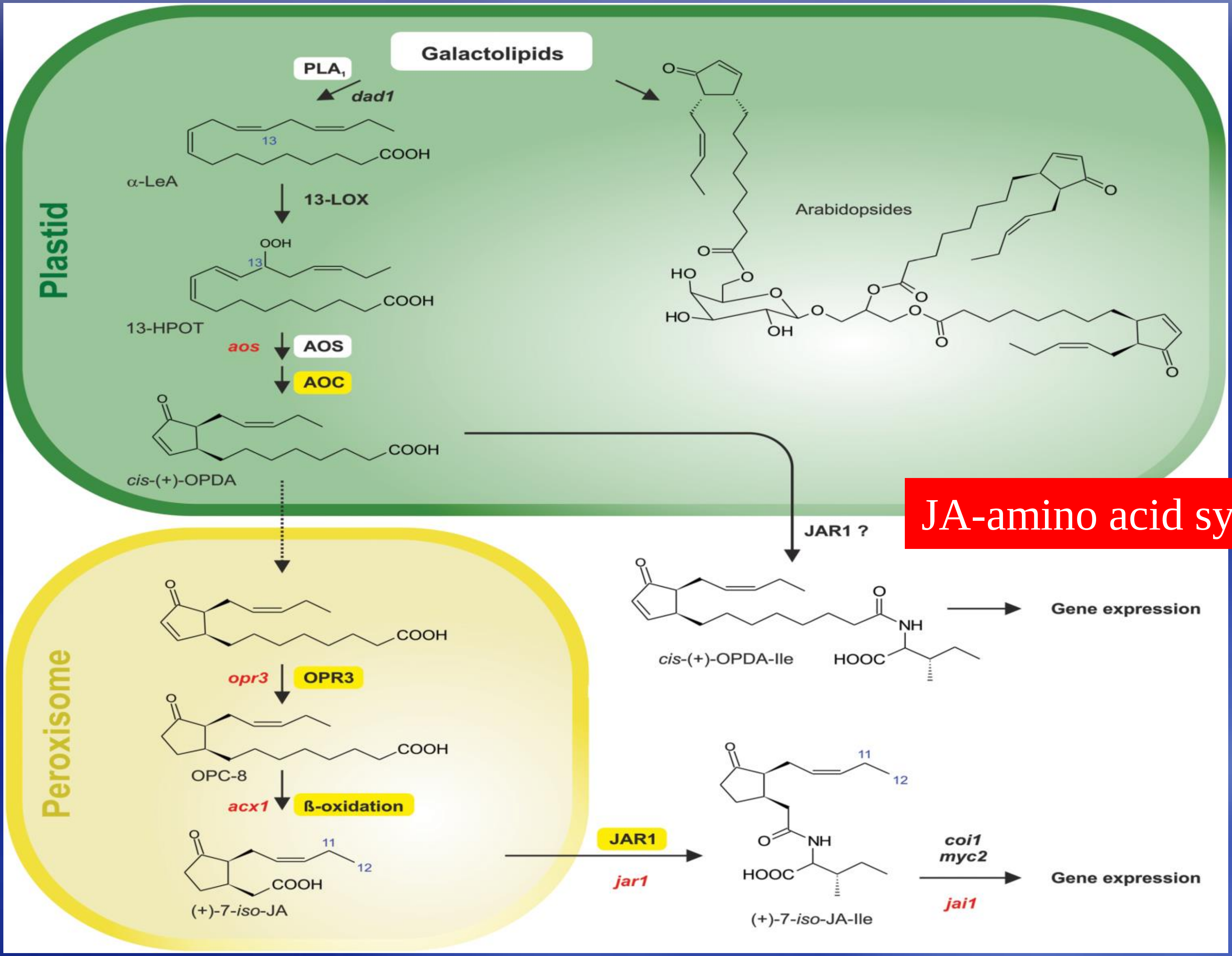
0 0.5 1 2 5 mM

Mature-green tomato fruit response to Colletotrichum quiescent



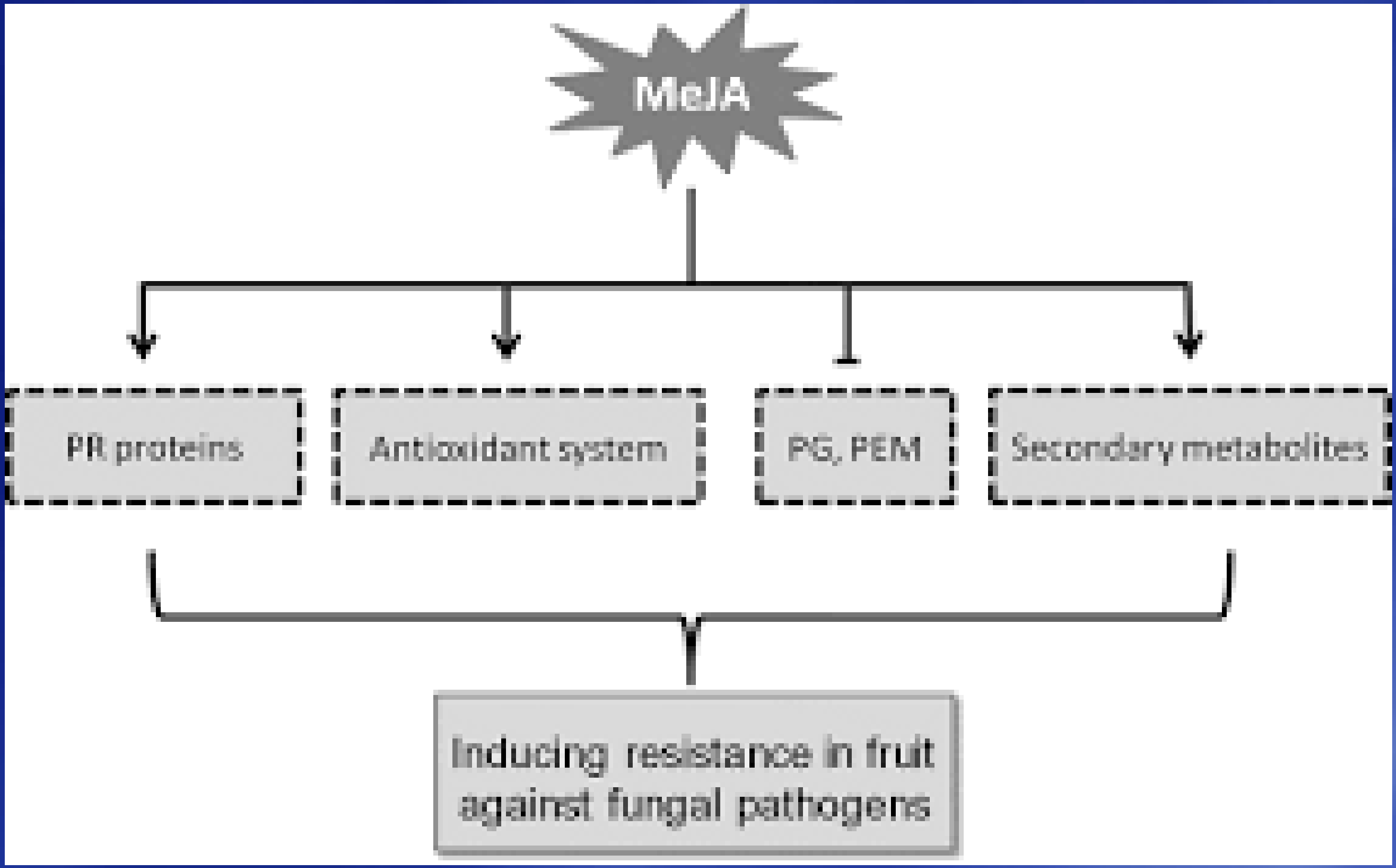
(Alkan et al., New Phytologist., 2015)

Synthesis of JA/JA-Ile from α-linolenic acid generated from galacto-lipids.

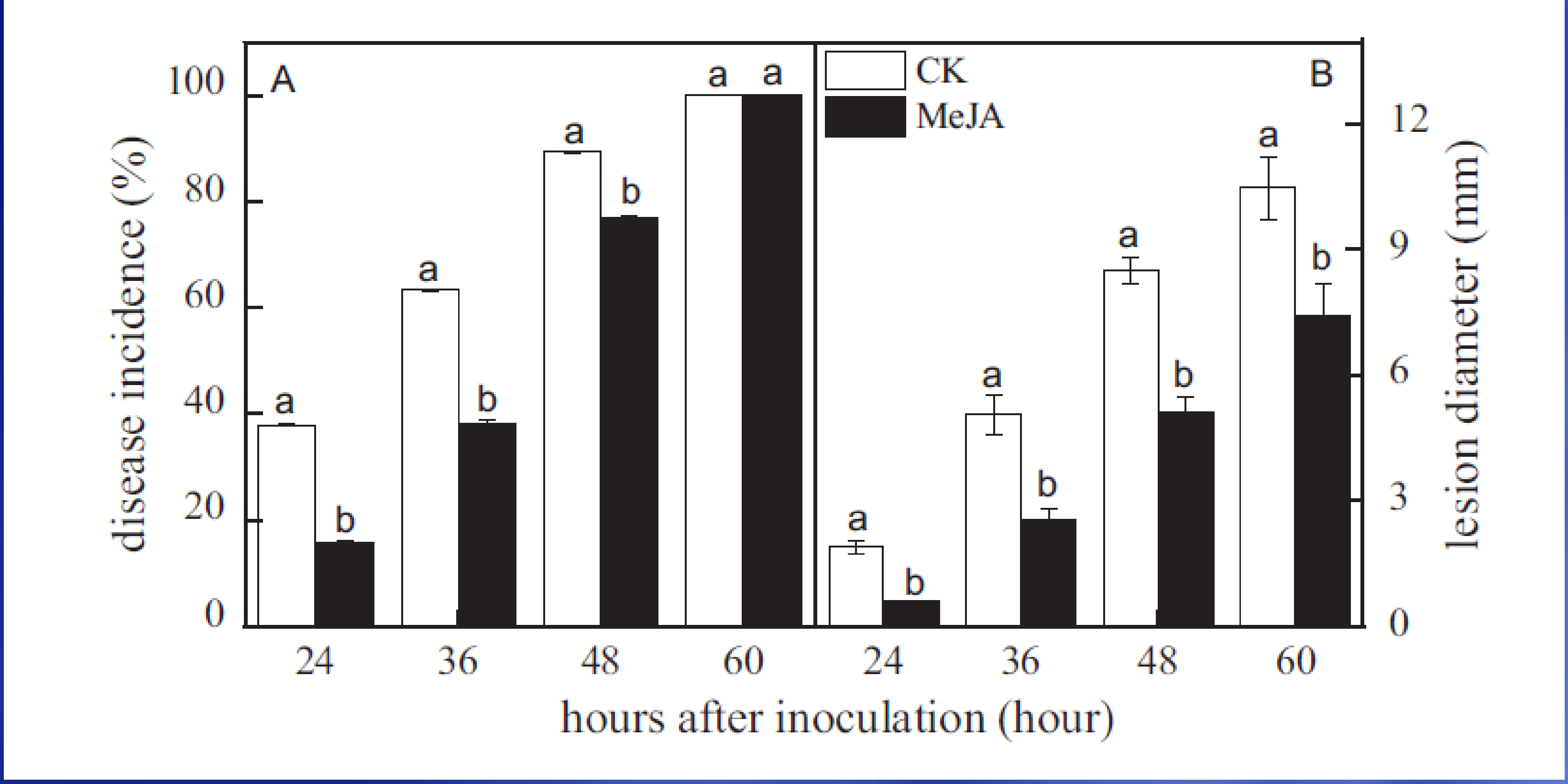


JA-amino acid synthetase;

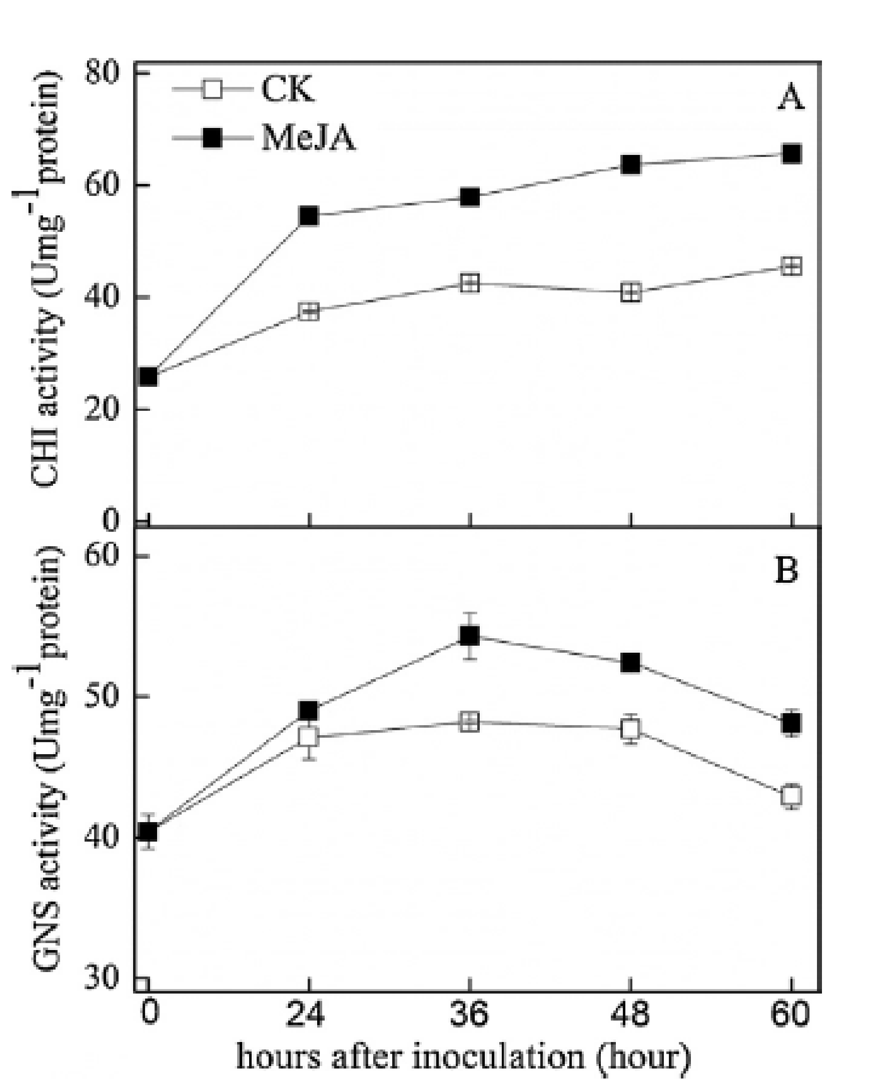
J Exp Bot, Volume 68, Issue 6, 1 March 2017, Pages 1303–1321, https://doi.org/10.1093/jxb/erw443


MeJA
PR proteins
Antioxidant system
PG, PEM
Secondary metabolites
Inducing resistance in fruit against fungal pathogens

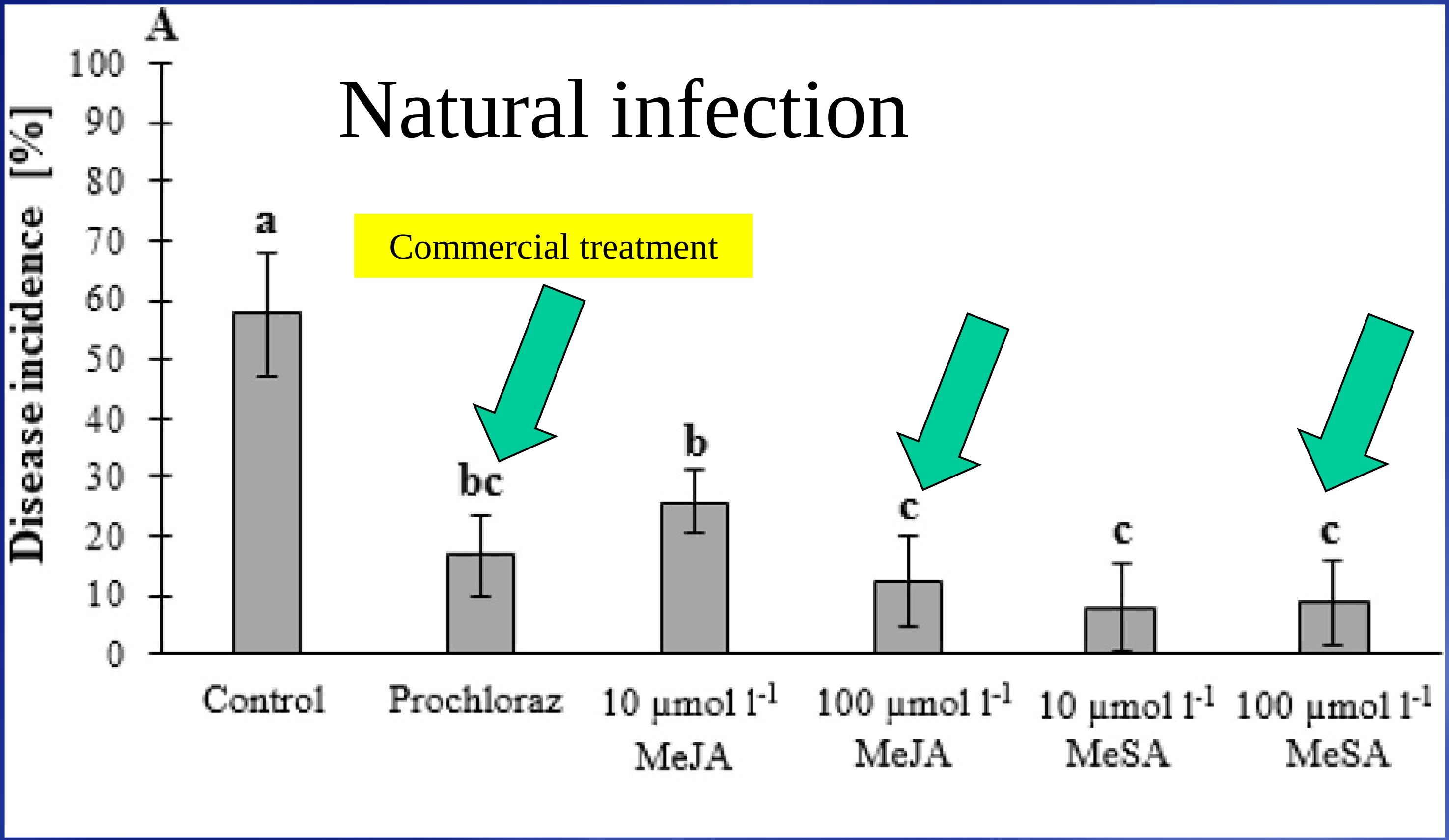
MeJA-induced resistance in harvested grapes inoculated by *Botrytis*



MeJA-induced the activation of pathogenesis-related proteins such as CHI (chitinases) and GNS (β-1,3-glucanases) in grapes inoculated by *Botrytis*



Anthracnose disease incidence in naturally infected ‘Hass’ avocado fruit treated with MeJA and MeSA



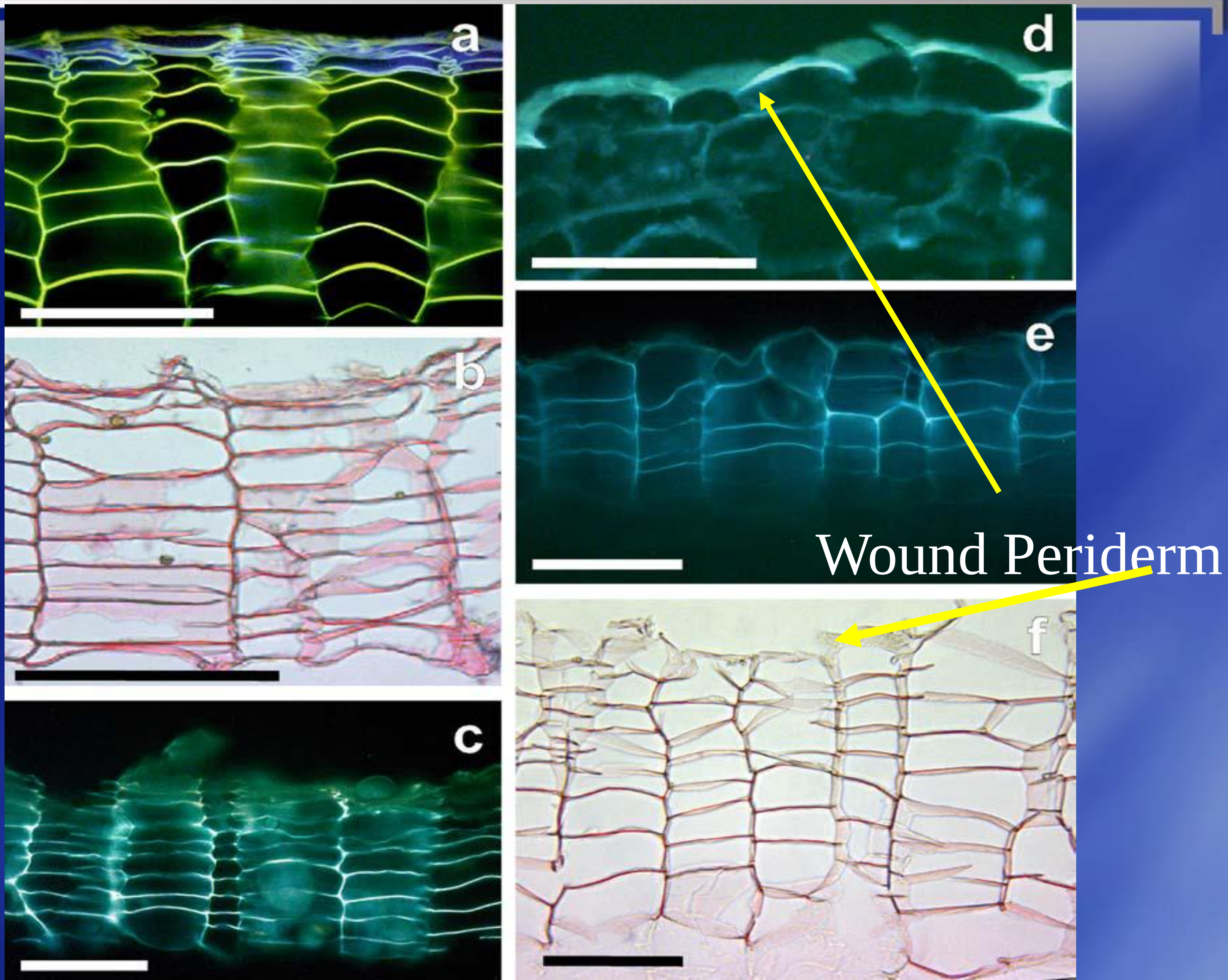
Fruit kept at 2 C for 14 d followed by 6–7 d shelf-life at 20 C

Induction of resistance of wounded tissue

Curing or wound healing of wounds

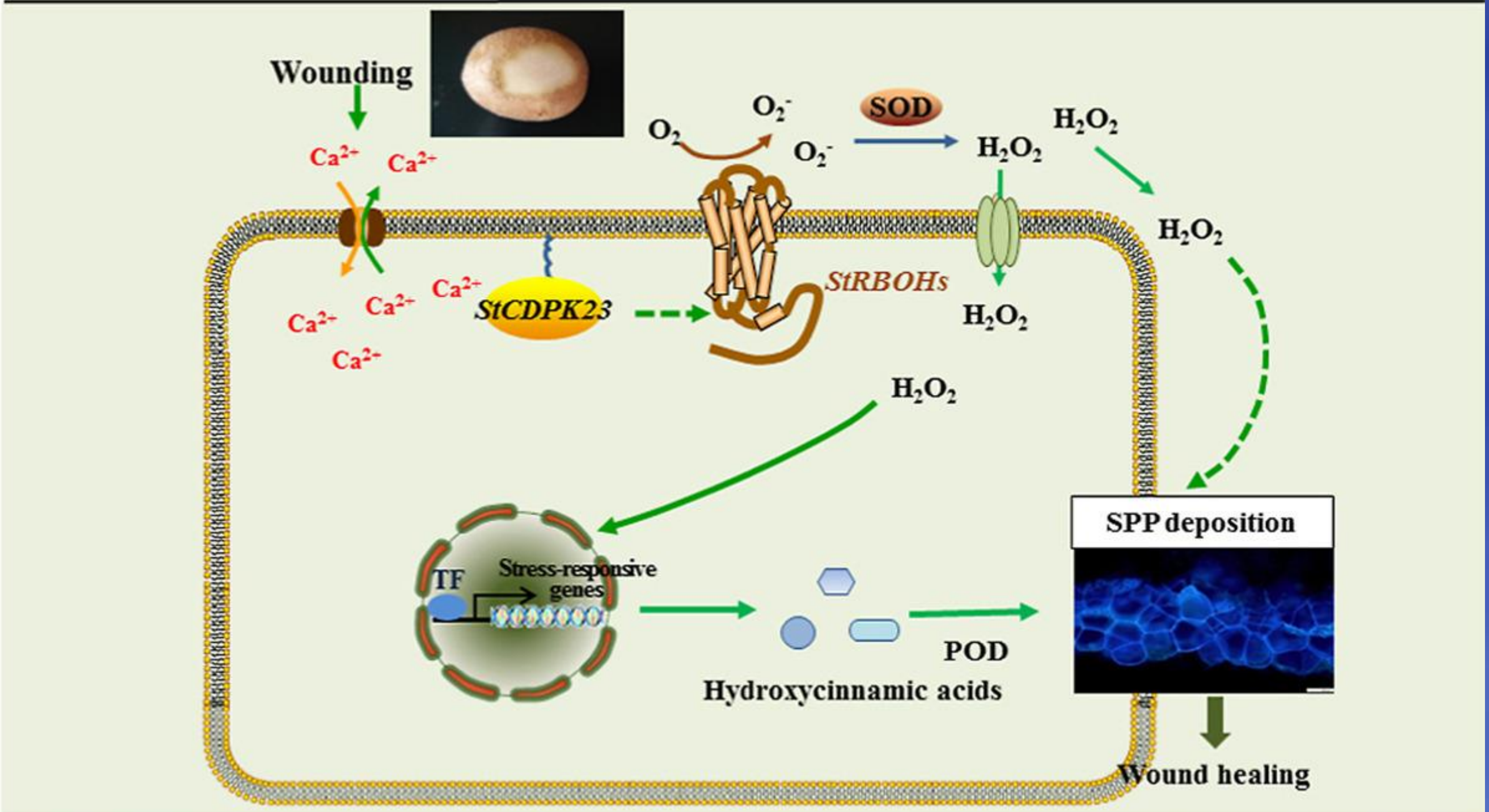
Under commercial conditions curing is carried out by storage of potatoes and sweet potatoes for about 10 days at 26-29 C degrees with high relative humidity of about percent. (80-85%)

Lead to the induction of wound-healing processes including deposition of suberin phenolic and aliphatic components and soluble waxes


a
b
c
d
e
f
Wound Periderm

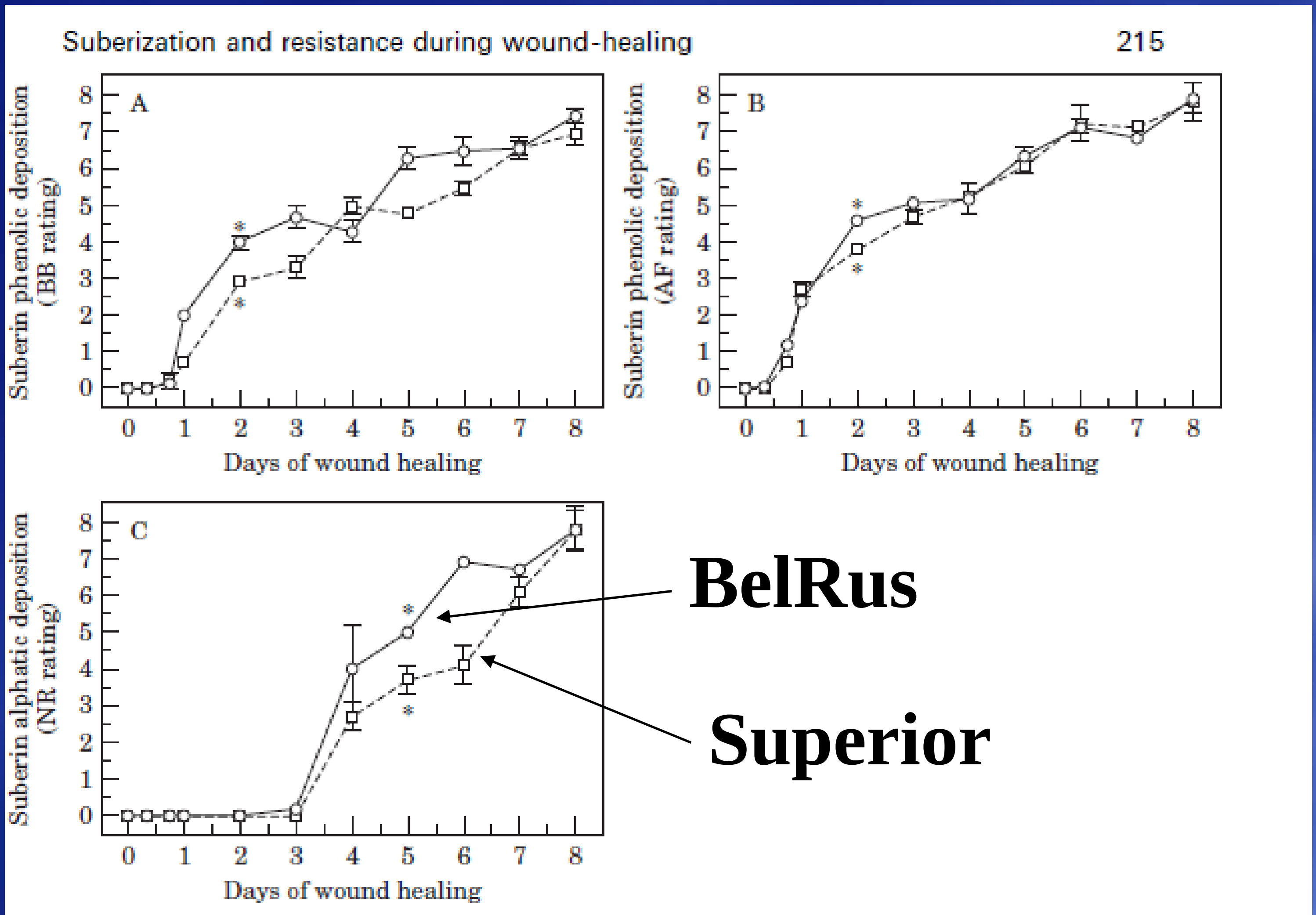
Ca Dependent Kinases

The mechanism of *StCDPK23* is involved in potato tuber wound healing



CONCLUSION: Wounding induced Ca^{2+} fluctuation, which acts as a signal to activate *StCDPK23*. *StCDPK23* is upstream regulators of *StRbohs* and activated Rboh-mediated O_2^- production by phosphorylation; O_2^- is converted into H_2O_2 via the action of SOD. H_2O_2 acts as a signal respond to wounding, and also as a cross-linking oxidant contributes to the healing of potato tuber.

Time course for deposition of suberin phenolic (A and B) and aliphatic (C) domains during wound-healing for cv. BelRus and Superior. At 5 days (*) after wounding, BelRus tubers became fully resistant while Superior tubers remained susceptible to *F. sambucinum* infection



Development of tuber resistance by curing

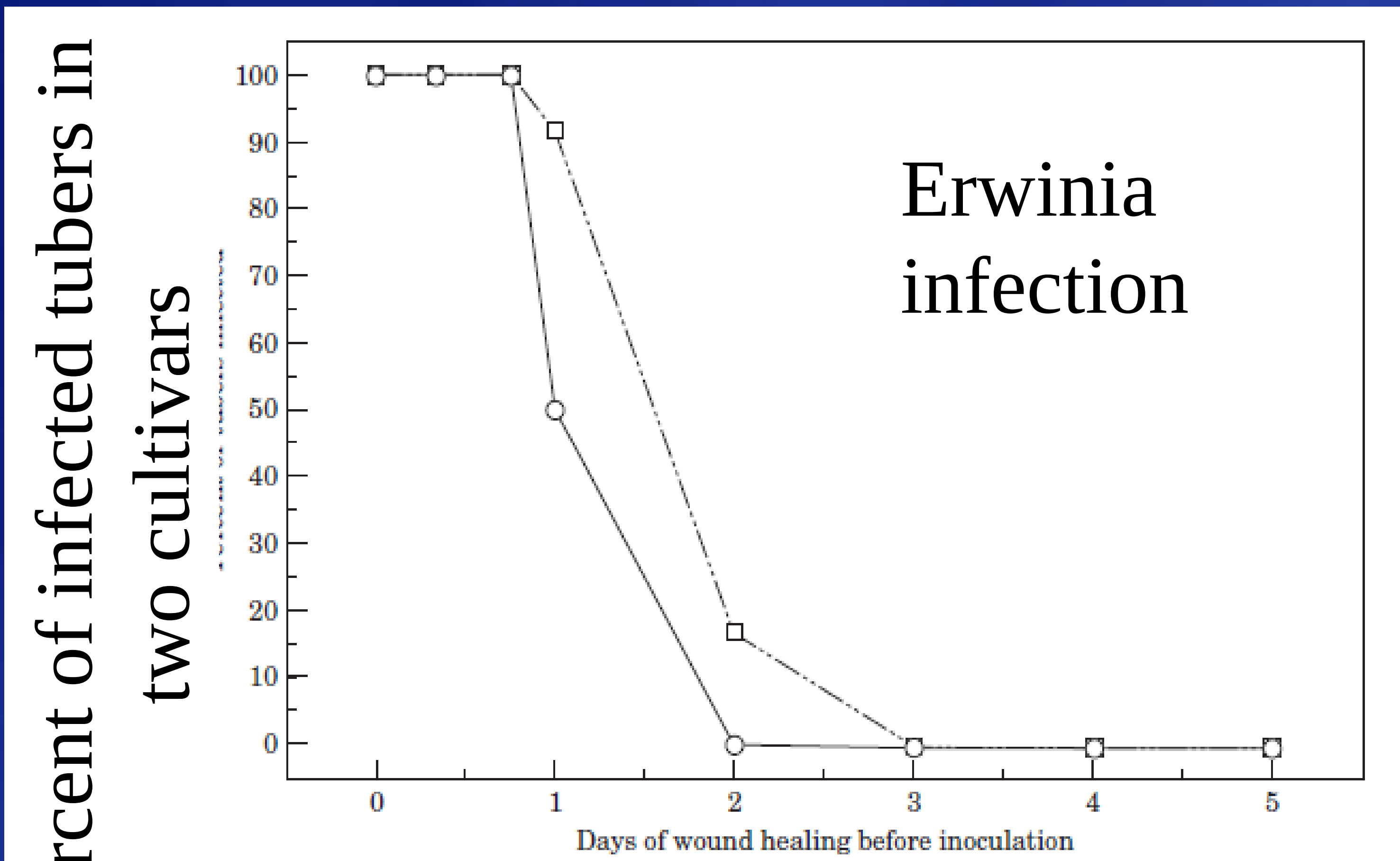


Fig. 2. Development of tuber resistance to infection by *E. carotovora* subsp. *carotovora* during wound-healing. The percentage of tubers infected was determined after incubating the inoculated tubers for 7 days at 18 °C and 98 % RH. Cultivars (○) BelRus; (□) Superior.

Development of tuber resistance by curing

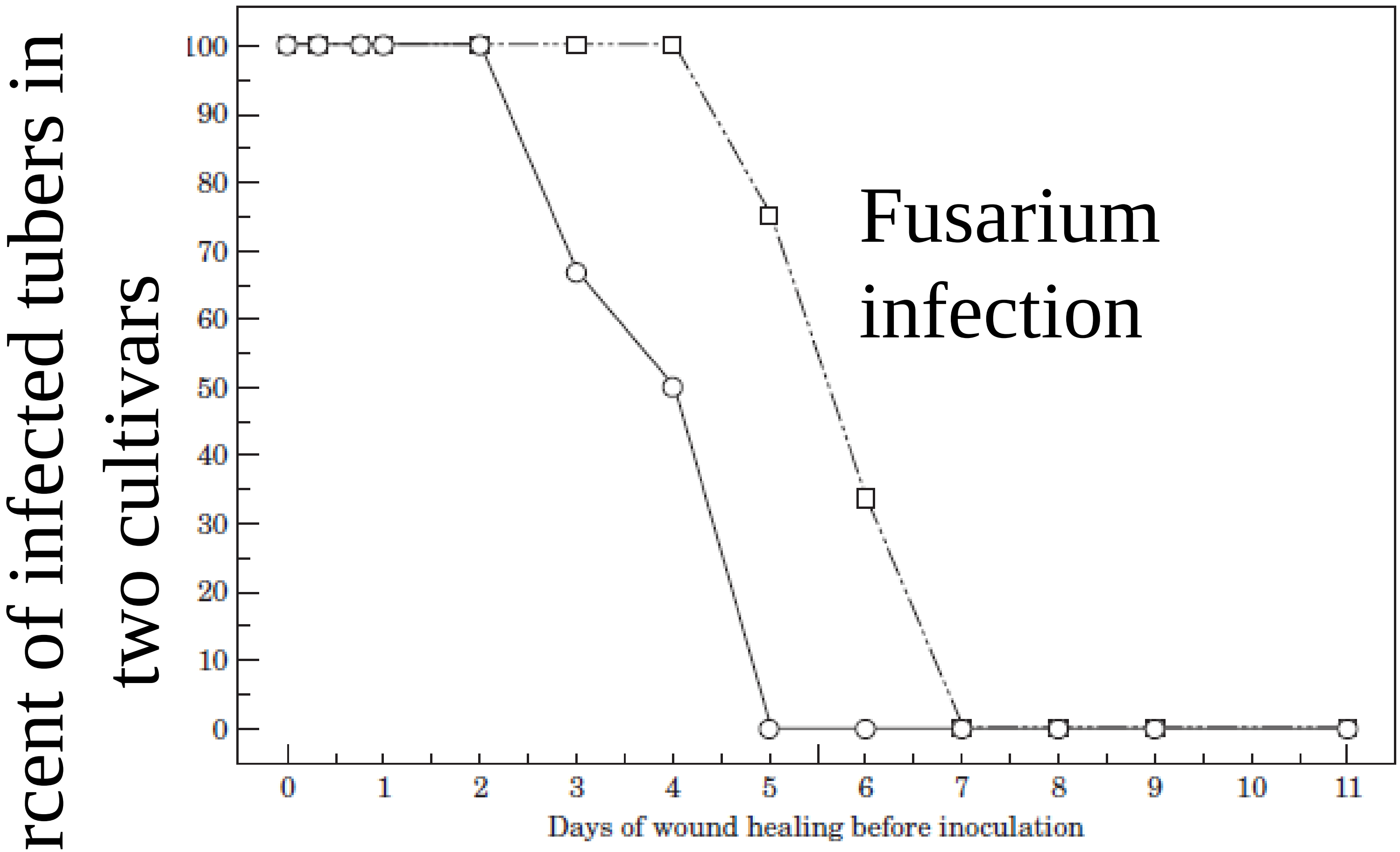


FIG. 3. Development of tuber resistance to infection by *F. sambucinum* during wound-healing. The percent of tubers infected was determined after incubating the inoculated tubers for 21 days at 18 °C and 98% RH. Cultivars (○) BelRus; (□) Superior.

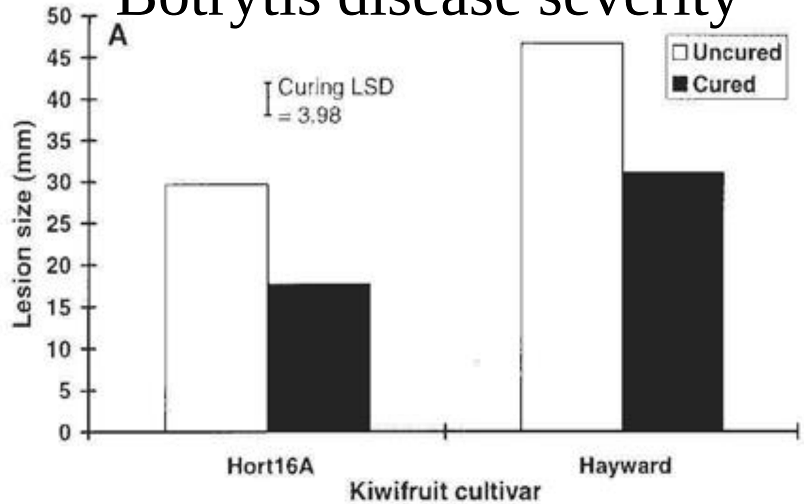
Curing in kiwi fruits

Measurement of defenses induced by curing was confined to phenolic and hydrolytic enzyme activities, since these appear to be key resistance markers in other *B. cinerea-plant* interactions.

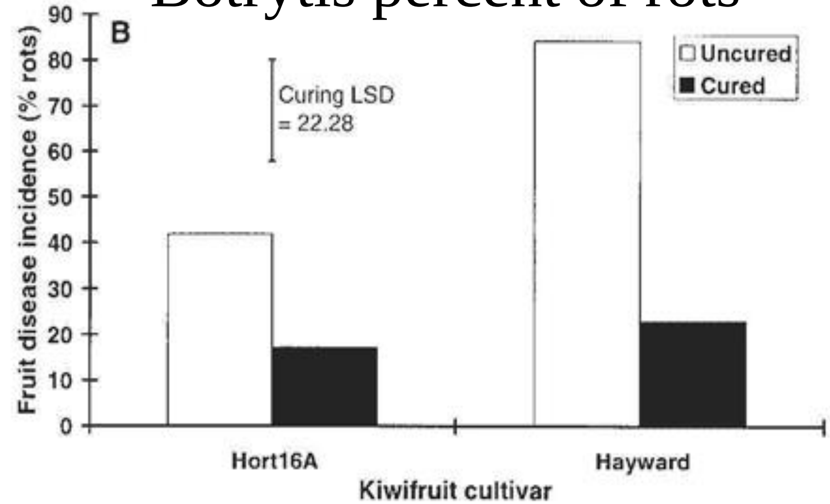
For the curing treatment, pedicels were removed by hand and fruit were cured for 1 week at 17°C to induce wound responses, followed by inoculation with *B. cinerea* at the end of the curing period

Curing in kiwi fruits

Botrytis disease severity



Botrytis percent of rots



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Summary of the mechanism of activation of quiescent infections

a. Histone modification in the pathogens
b. Preformed host antifungal compounds
c. Preformed host antifungal enzymes
d. Inducible host antifungal compounds
e. Inducible ROS by the host
f. Inducible ROS by the fungus
e. Inducible phenyl propanoic metabolism in the host wounds