2nd Lecture
Resistance and susceptibility in harvested fruits

Pathogenicity, resistance and susceptibility

- Host factors modulating postharvest pathogen development
- Resistance mechanisms in fruits

The interaction between the fruit host and the pathogen

- Interaction of a single gene
- Interaction of several genes that are modulated by fruit development and ripening

Dynamic interaction

Defense in fruit pathogen interactions

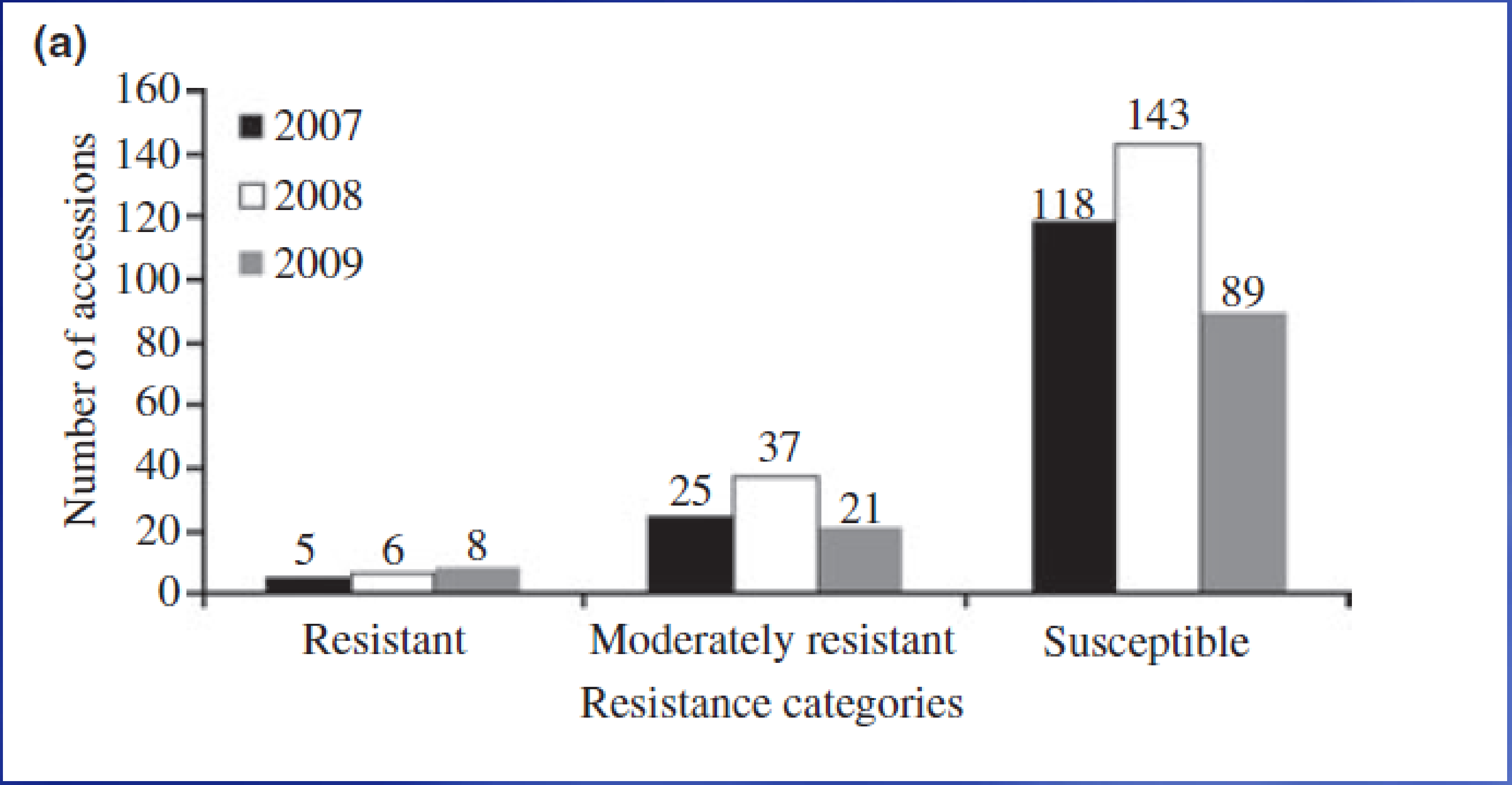
1. **Basic defense lines**
2. **Hierarchical response line during colonization**

Defense in fruit pathogen interactions

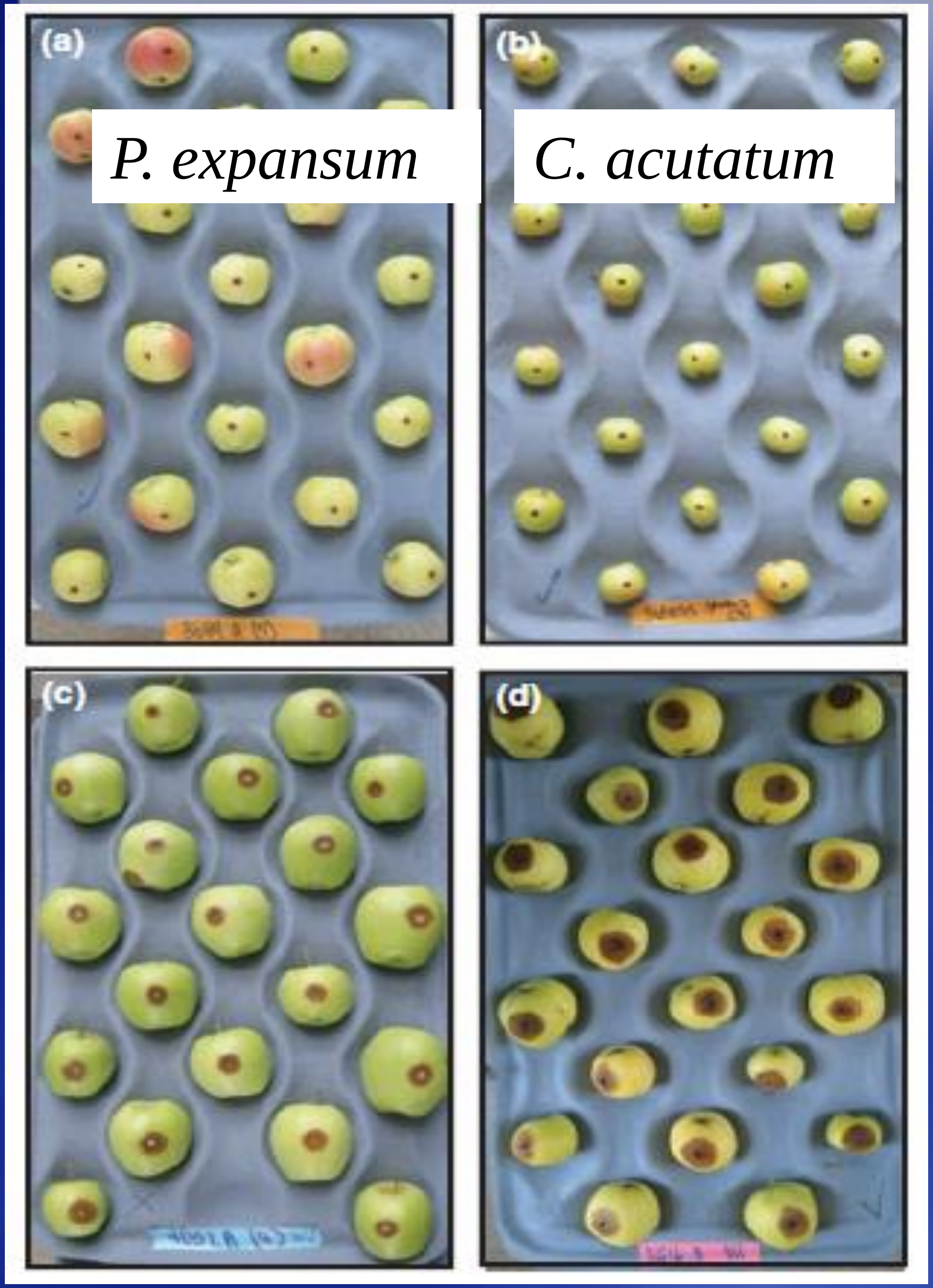
1. **Basic defense lines**

Are defense mechanisms found in wild apple germplasm that show a higher level of gene response to fungal attack

Identification of wild apple germplasm (*Malus* spp.) accessions with resistance to the postharvest decay pathogens *Penicillium expansum*



Jurick et al. , 2011; Janisiewicz et al., 2008

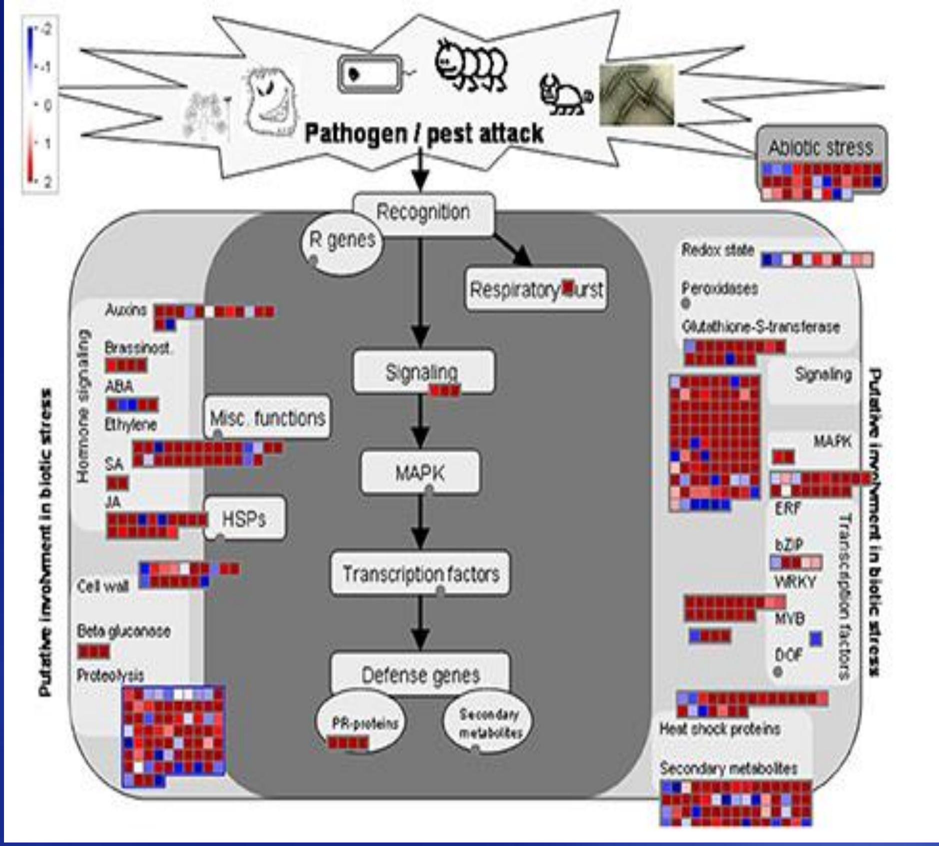


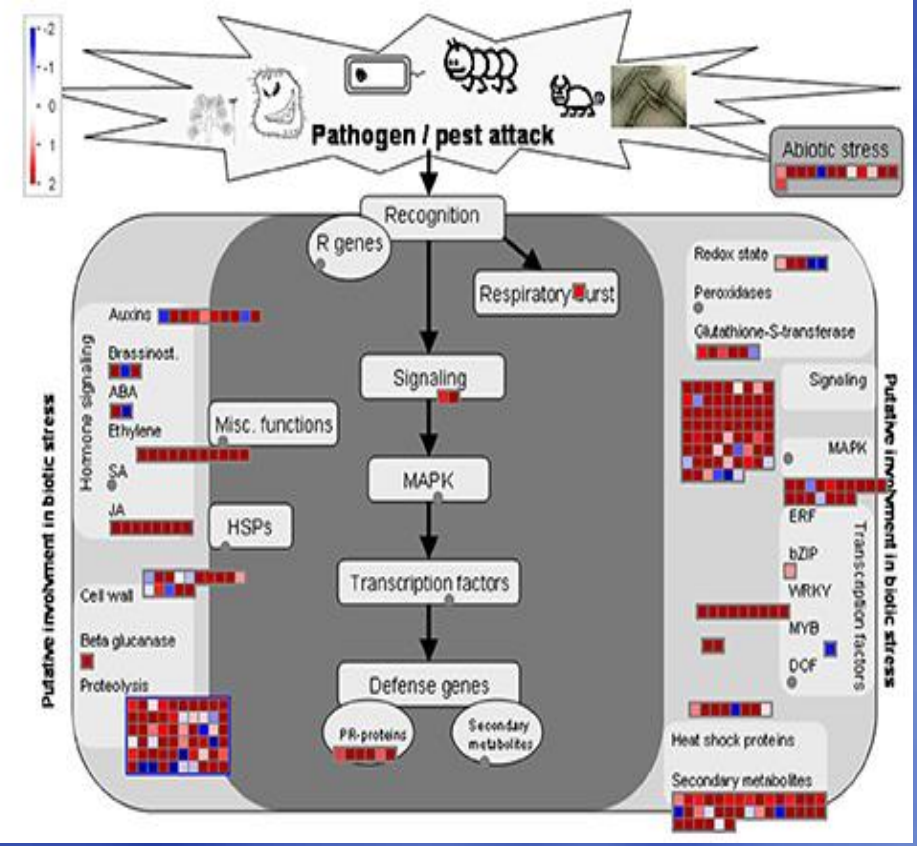
Differential susceptibility of several accession apples

Overview of genes related to biotic stress upon *P. expansum* infection after 6 hpi in the resistant vs. time 0. CHO, carbohydrates; OPP, oxidative pentose phosphate; TCA: tricarboxylic acid cycle.

Resistant apple line

Susceptible apple line





Transcriptomic analyses of resistance wild type phenotype after inoculation show:

1. A higher basal level of gene response,
2. A faster and intense defense response to wounding and wounding plus inoculation with *P. expansum* than the Royal Gala

Basic defense lines

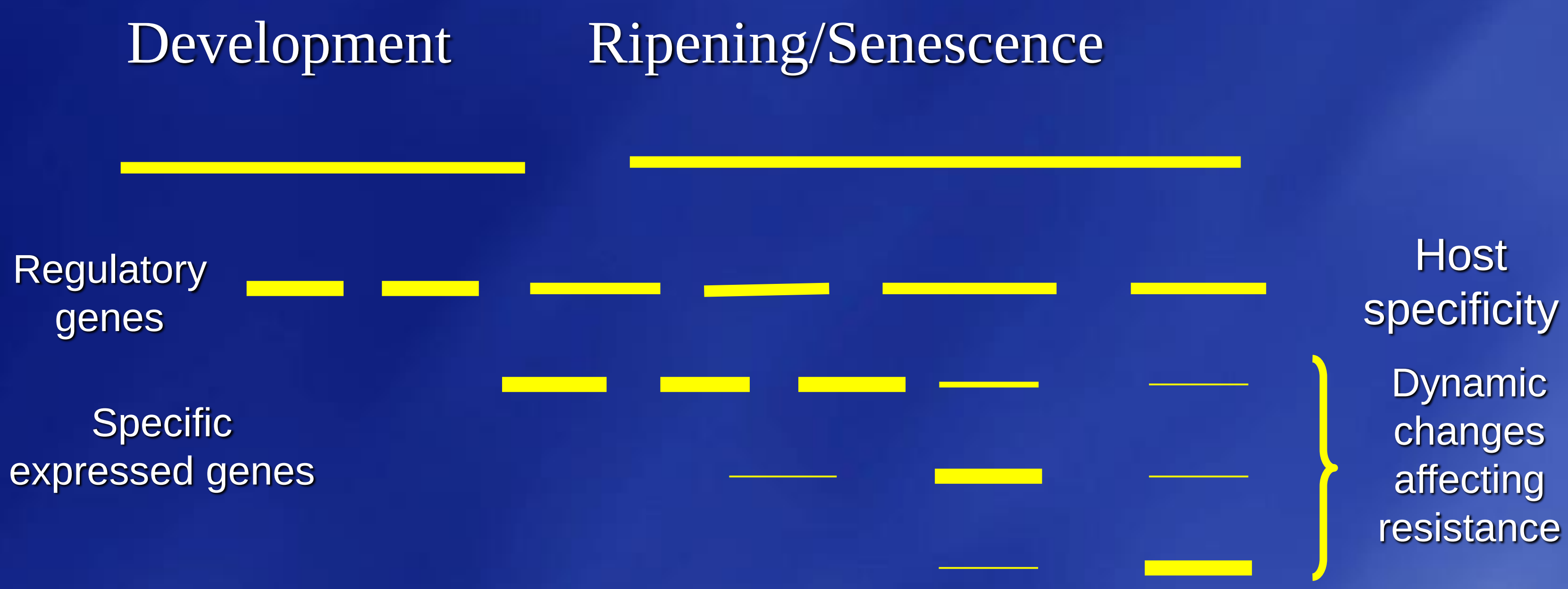
Ballester A-R, Norelli J, Burchard E, Abdelfattah A, Levin E, González-Candelas L, Droby S and Wisniewski M (2017) Transcriptomic Response of Resistant (PI613981–Malus sieversii) and Susceptible (“Royal Gala”) Genotypes of Apple to Blue Mold (Penicillium expansum) Infection. Front. Plant Sci. 8:1981. doi: 10.3389/fpls.2017.01981

Defense in fruit pathogen interactions

1. **Basic defense responses**

2. **Hierarchical response line during penetration and colonization resulting from a multigene interaction**

Mechanisms of host responses in fruit pathogen interactions



Resistant Fruit
Fungal Quiescence

Susceptible Fruit
Active infection

The hierarchical response is dependent:

On the dynamics of responses during the maturity of the crop, wounding responses and senescence

The fruit will continue to be resistance:

- If the mechanism involved in resistance is still present
- If the induced mechanism of resistance is still active
- If a new mechanism become activated (curing, phenol concentration, etc)

Conclusion: the hierarchial defense response in fruit pathogen interactions

- Is a complex and multicomponent factor and not conditioned by a single gene
- Defense response is mainly determined by the maturity and ripeness of the host
- The defense response in ripening fruits

  is a dynamic response on the same host

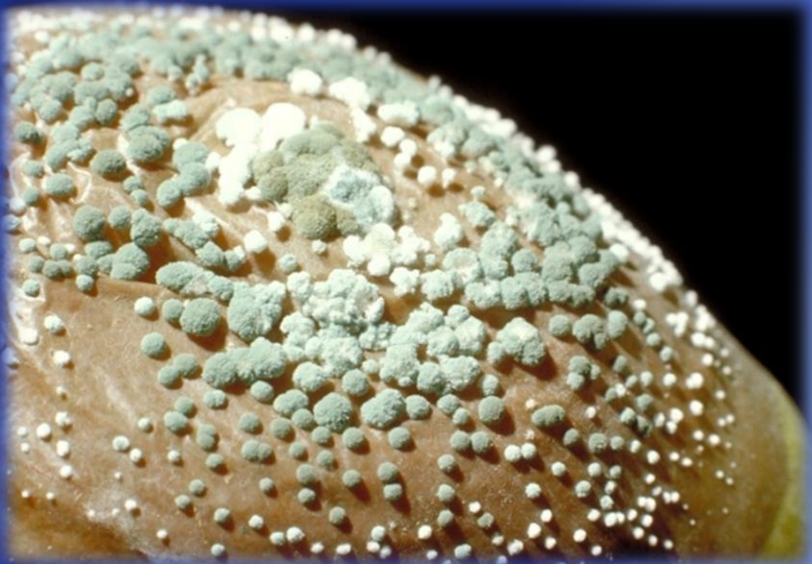
Mechanisms of colonization of postharvest fruit pathogens

- Wound, opportunistic colonization

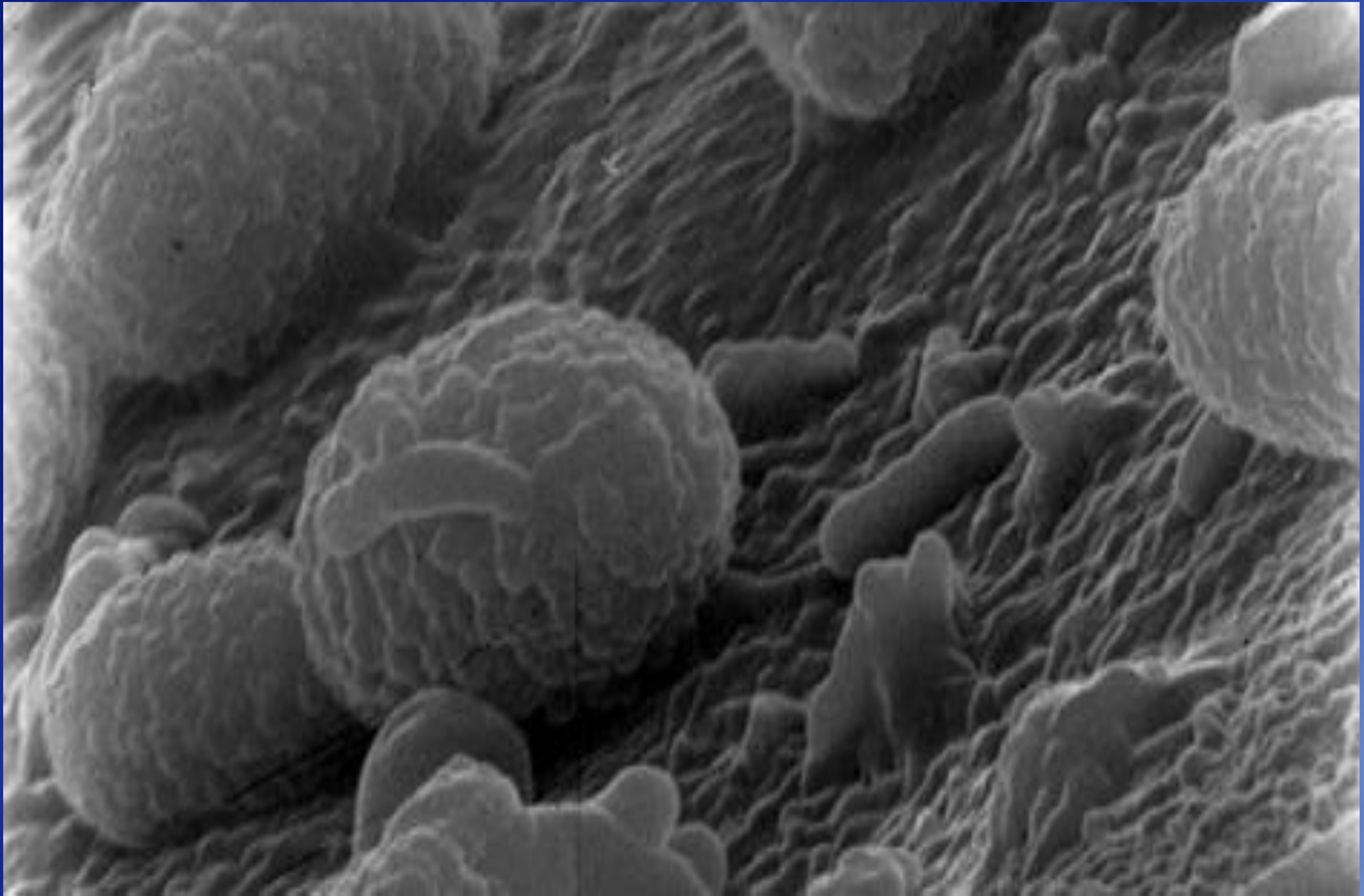
- Direct, delayed colonization
  Quiescent infecting pathogens

Mechanisms of fruit response to direct and wound pepentrating pathogens

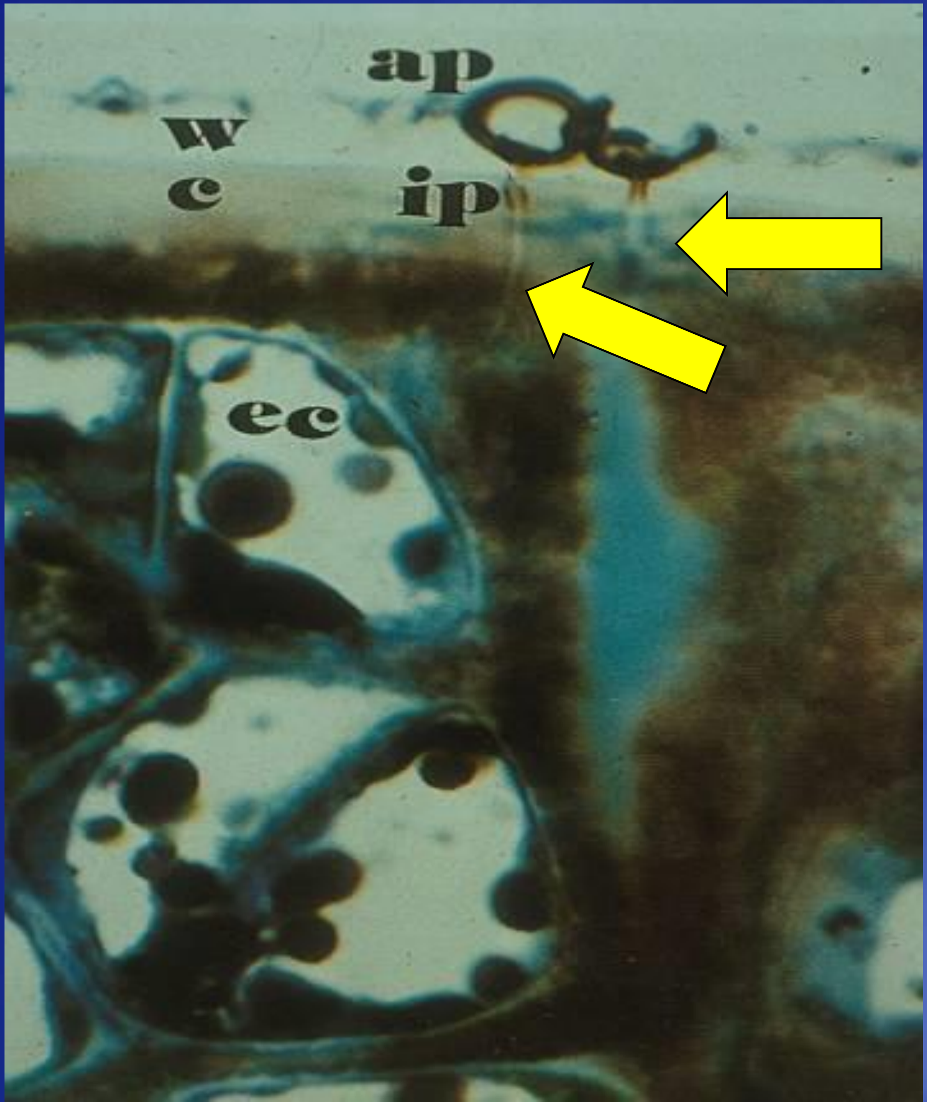
Penicillium expansum colonization



Colletotrichum gloeosporioides germination



Appressoria penetration and quiescence by Colletotrichum



Are the mechanism induced by host different for pathogens with direct or wound penetration?

Host responses induced against pathogen at different fungal developing stages”

Host response during:

- Spore germination
- Appressoria formation
- Germination of appressoria
- Effect of colonization

Host responses induced against wound pathogens during: spore germination

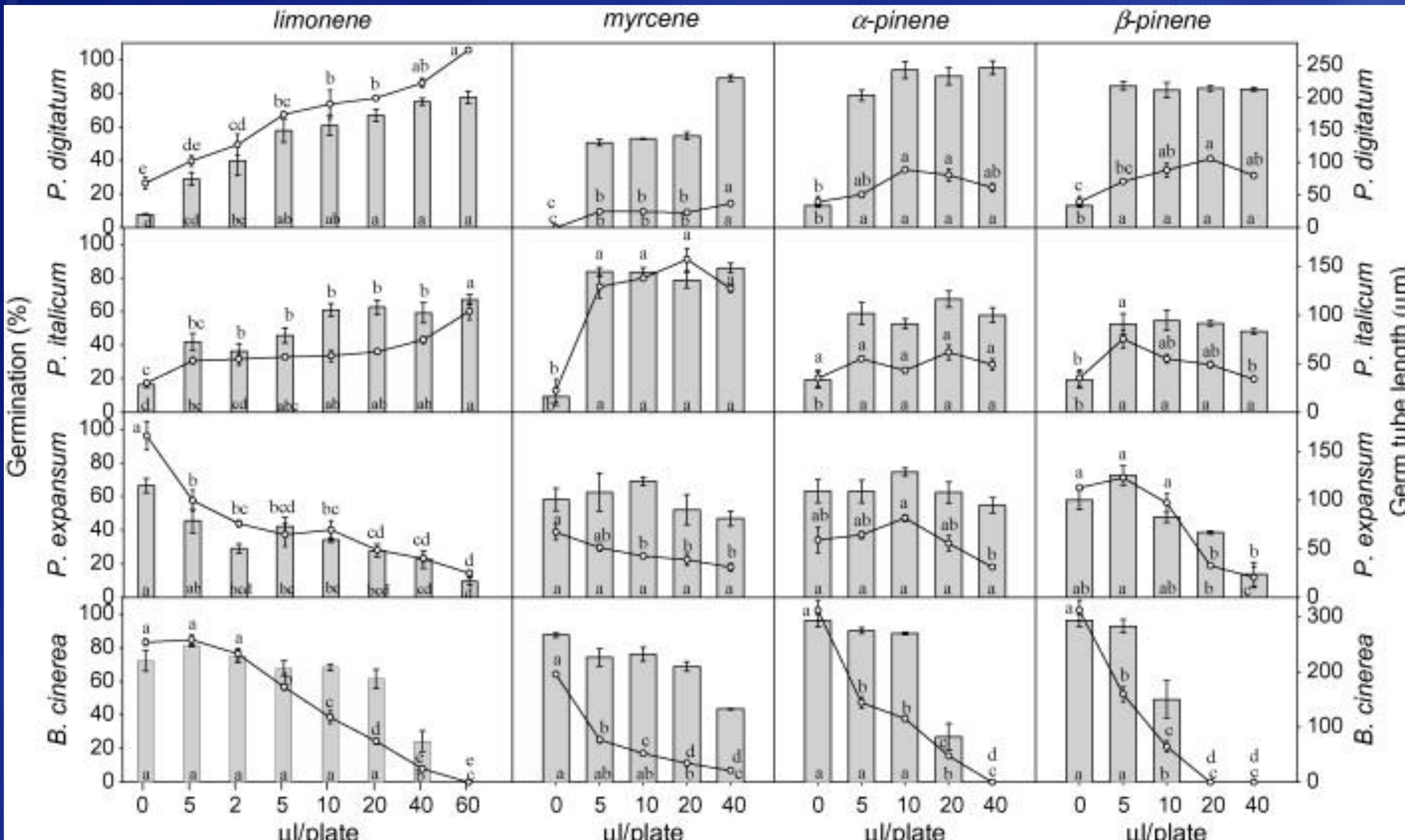
- Host-wound-volatiles monoterpenes affect germination and fungal colonization

Concentration of constituent volatiles from the head space of wounded clementine, orange and grapefruit

Compound/oil	Clementine	Orange	Grapefruit
α-Pinene	1.25	0.71	0.80
Sabinene	0.21	0.39	0.71
Myrcene	2.33	2.12	2.31
Octanal	0	0	0.40
Limonene	88.89	86.80	92.34
γ-Terpinene	4.76	0	0
β-Ocimene	0	0	0.17
Linalool	0.54	0.56	0.10
Decanal	0.10	0.26	0.33
Caryophyllene	0	0	0.43
Nootkatone	0	0	0.37

Role of citrus volatiles in host recognition, germination and growth of Penicillium digitatum and Penicillium italicum. 2008. Droby et al.

Effect of the volatile monoterpenes of wounded fruit, limonene, myrcene, α-pinene, and β-pinene on percent germination and growth (-○-) of *P. digitatum*, *P. italicum*, *P. expansum* and *B. cinerea*.



It can be concluded

Volatiles monoterpenes of wounded citrus fruit may specifically contribute germination and pathogenicity of *Penicillium* and inhibit germination of *Botrytis*

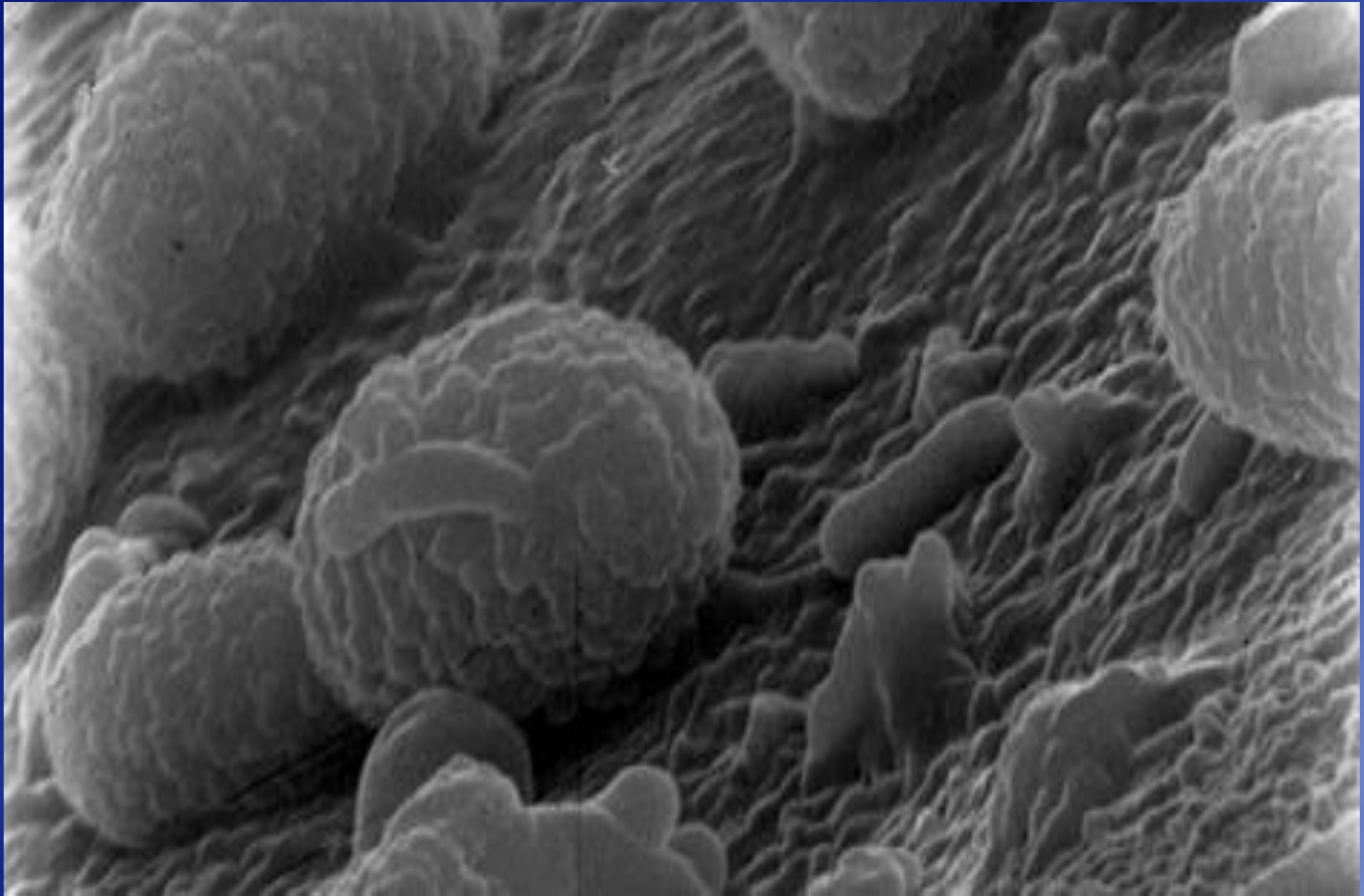
Host responses induced to direct penetration pathogens

- Effect of appressoria formation (infection peg) and germination

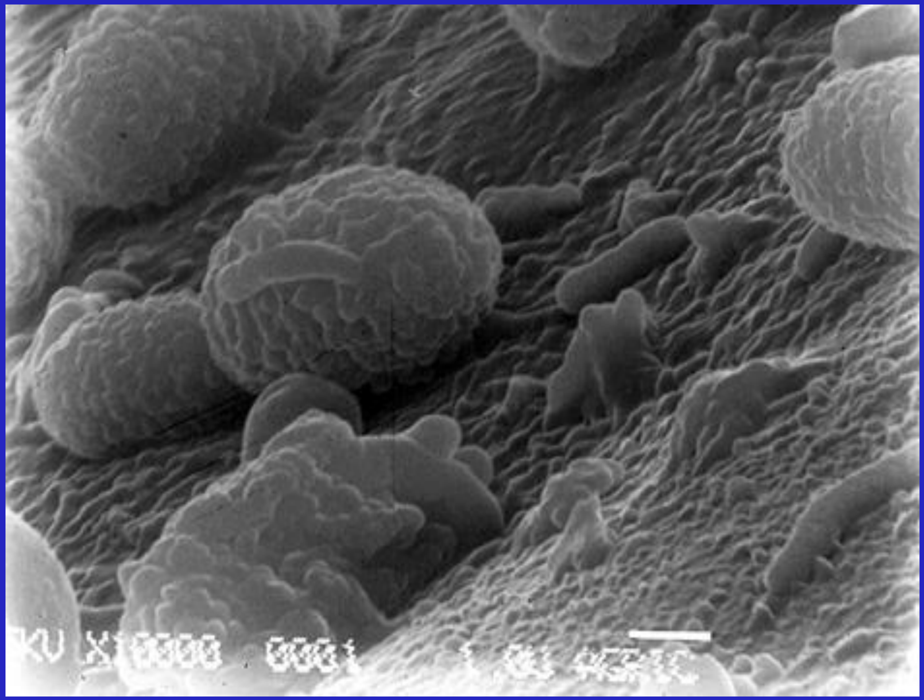
Nuclear division and morphogenesis during early stages of pathogenic germination of *Colletotrichum*

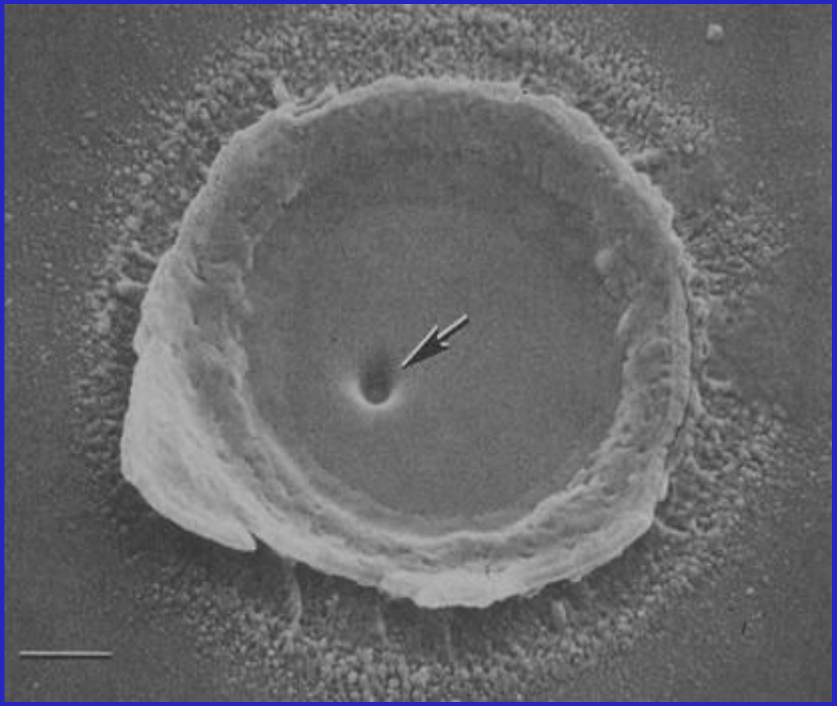
(a) resting spore; (b) first nuclear division (75–90 min); (c) germ tube formation (90–120 min); (d e

Colletotrichum gloeosporioides germination on avocado peel



Colletotrichum direct penetration and appressorium formation





Is the fruit host an inducer or an inhibitor for of appressoria formation to initiate fruit attack?

Host responses induced to direct penetration pathogens

1. **Secretion anthranilic acid by banana and transformation to 2,3 dihydroxy benzoic acid by *Colletotrichum* induces appressoria formation**

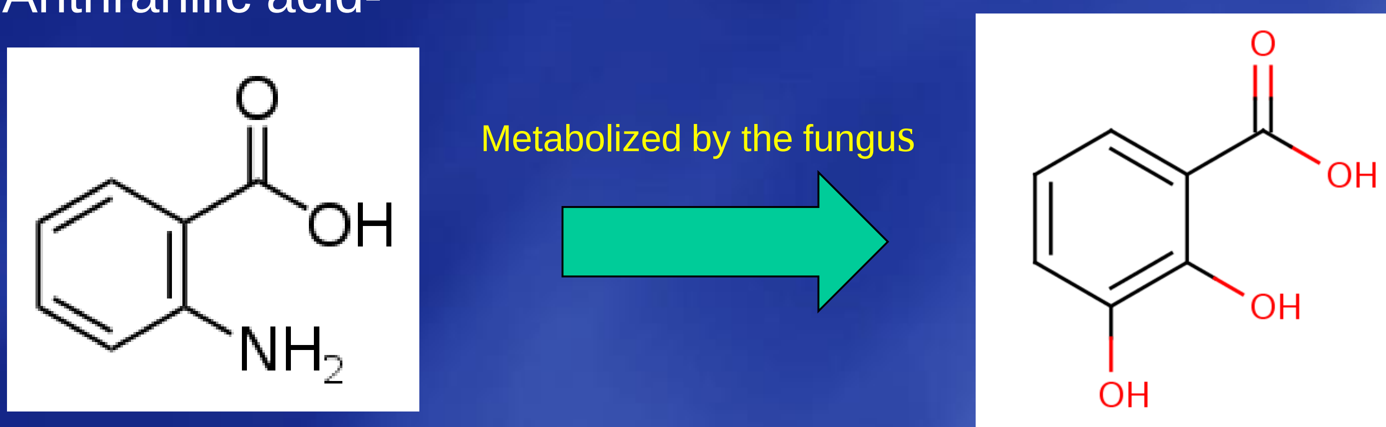
Exuded by the banana

Stimulate appressoria formation

Anthranilic acid- → **2,3 dihydroxy benzoic ac**

Anthranilic acid an iron binding compound, named 2-aminobenzoic acid is metabolized by the fungus to 2,3 dihydroxybenzoic acid and stimulated appressoria formation by *C. musae*

Anthranilic acid-



2-aminobenzoic acid

2,3 dihydroxy benzoic ac.

Similarly leachates of the apple cv. “Bramelys Seedlings” contain chlorigenic acid and p-cumaryl quinic acid both stimulate germination and appressoria formation of the apple pathogen *Phomopsis mali*

(Diaporthe perniciosa)

Host responses induced to direct penetration pathogens

1. **Secretion anthranilic acid by banana and transformation to 2,3 dihydroxybenzoic acid by *Colletotrichum* induces appressoria formation**

2. **Host wax modulate conditions for appressoria formation**

Effect of Various Plant Waxes on Appressorium Formation by *C. gloeosporioides*

Assays were done by the coated cover glass cover with wax

Source of Wax	Appressoria (%)
Avocado fruit	78
Broccoli leaves	1
Cabbage leaves	4
Pea leaves	4
Jade leaves	4
Sweet potato tuber	6
No wax	3

Effect of Avocado Wax on Appressoria Formation by various Colletotrichum spp. Pathogens

Pathogens	Host	Appressoria formation (%)
C. gloeosporioides	Avocado	70
C. trifolii	Alflalfa	0
C. orbiculare	cucumber, watermelon	2
C. pisi	Peas	0
C. capsici	Cotton, peppers	0
C. lindemuthianium	Bean	70
C. coccodes	Tomato	0

Appressorium-Inducing Activity of Various TLC Fractions from avocado wax on *C. gloeosporioides*

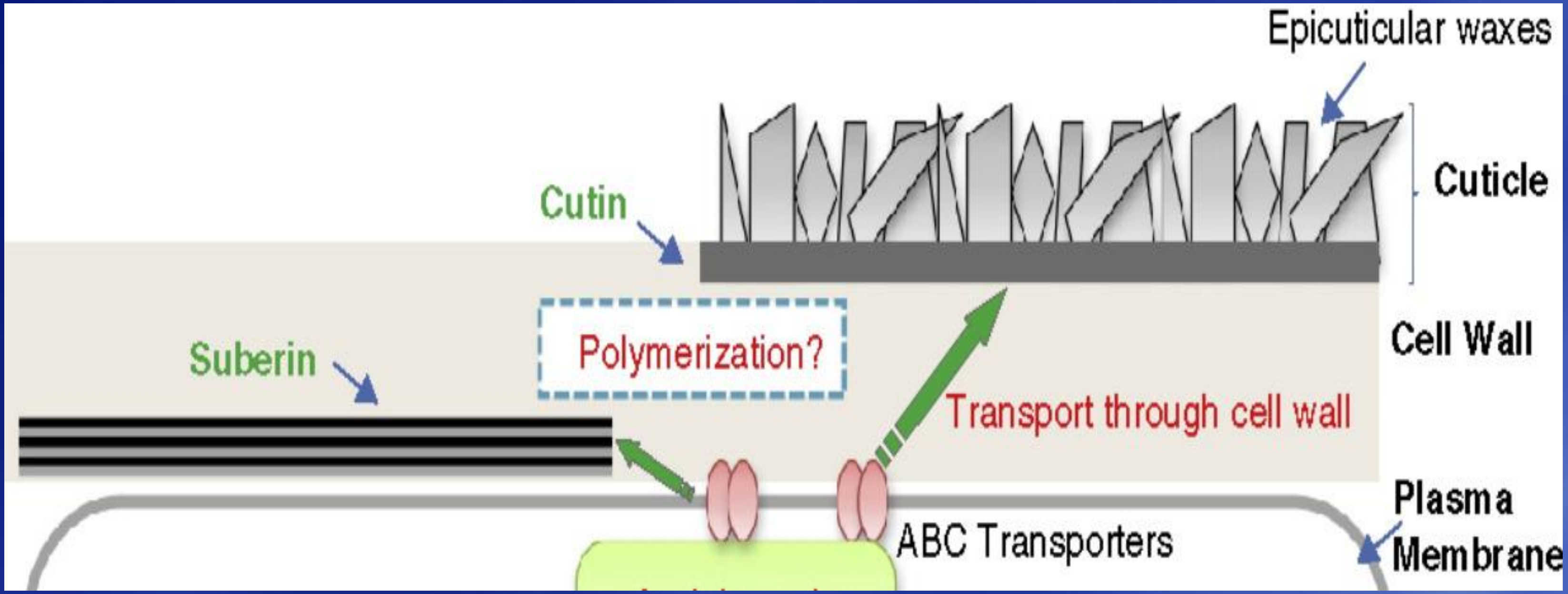
Fraction	Appressorium formation %	Wax Composition % of wax by weight
Hydrocarbon	1	62
Wax ester + ketone	7	6
Aldehyde	11	7
Secondary alcohol	23	4
Fatty acid	70	4
Primary alcohol	95	5
Origin	74	11

Effect of Addition of Other Plant waxes on Appressorium Formation Induction in *C. gloeosporioides* by Avocado Wax

The percentage of appressoria formed with avocado wax alone (74%) was used as the control value.

Addition	Amount mg	Appressorium induction % of control
Broccoli wax	0.5 2.0	14 19
Odoris wax	2.0	27

Not only the epicuticular wax but the cutin wax contribute to germination and penetration to fruits

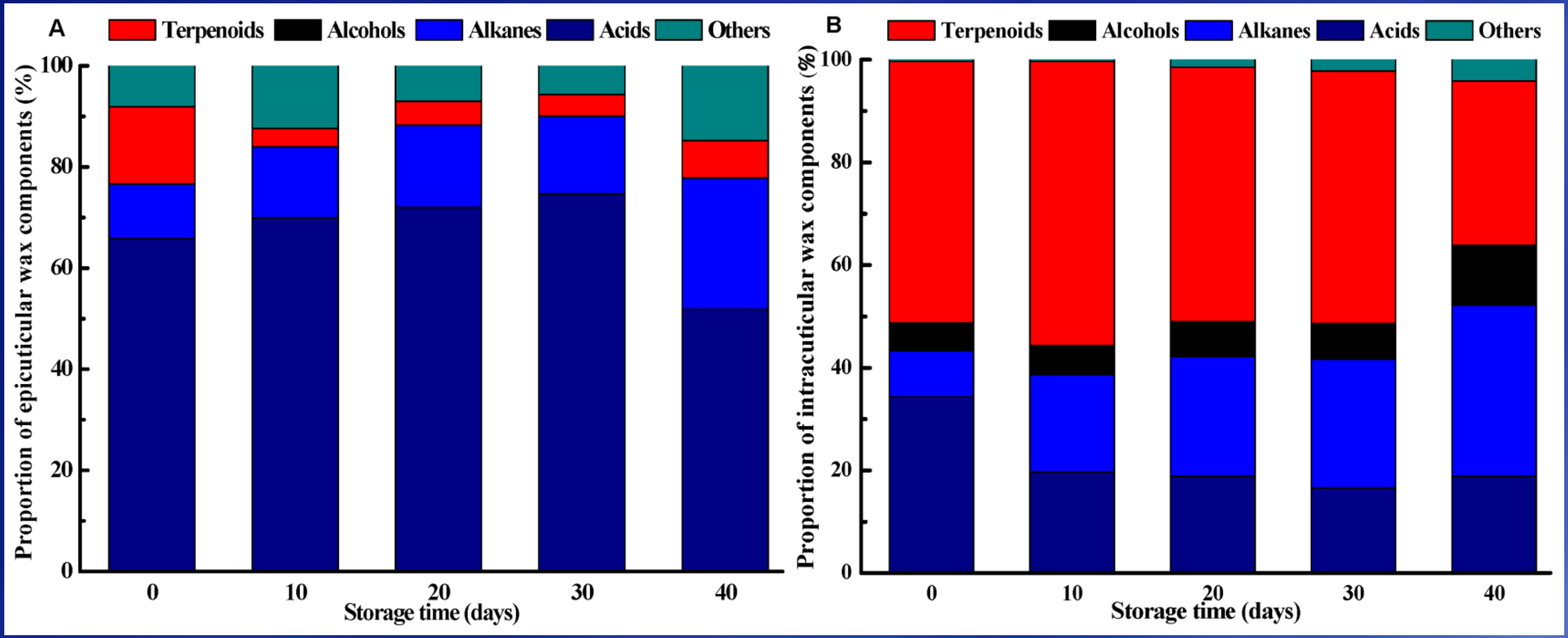


Cuticle components

- **<u>Epicuticular wax</u>** (i.e. wax exterior to cutin that can be mechanically peeled off)
- **<u>Intracuticular wax</u>** (i.e. wax residing within the mechanically resistant layer of cutin)

Molecules 2020, 25, 412; doi:10.3390/molecules25020412

Epicuticular wax components proportion (A) and intracuticular wax components proportion (B) of the '**Satsuma' mandarin** fruits during storage



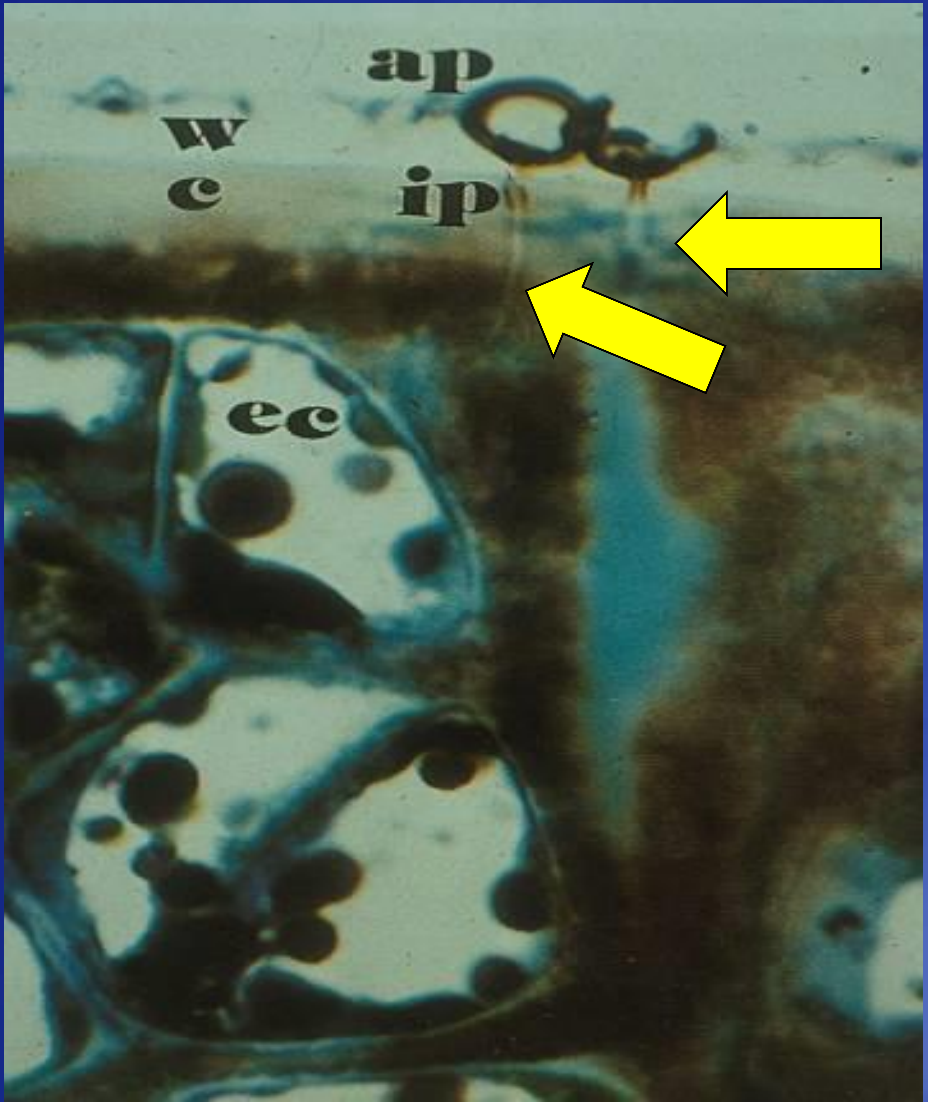
Results of in vitro tests showed that **mycelial growth** of *Penicillium digitatum* could be promoted by epicuticular wax and **conidial germination** could be inhibited by the cutin of the intracuticular wax at different storage stages.

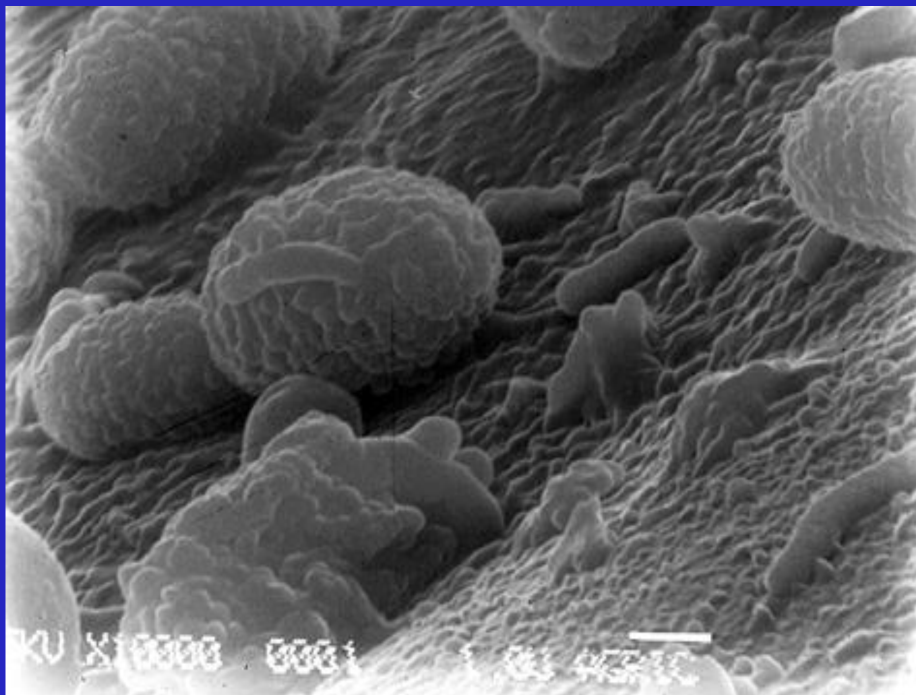
Host responses induced to direct penetration pathogens

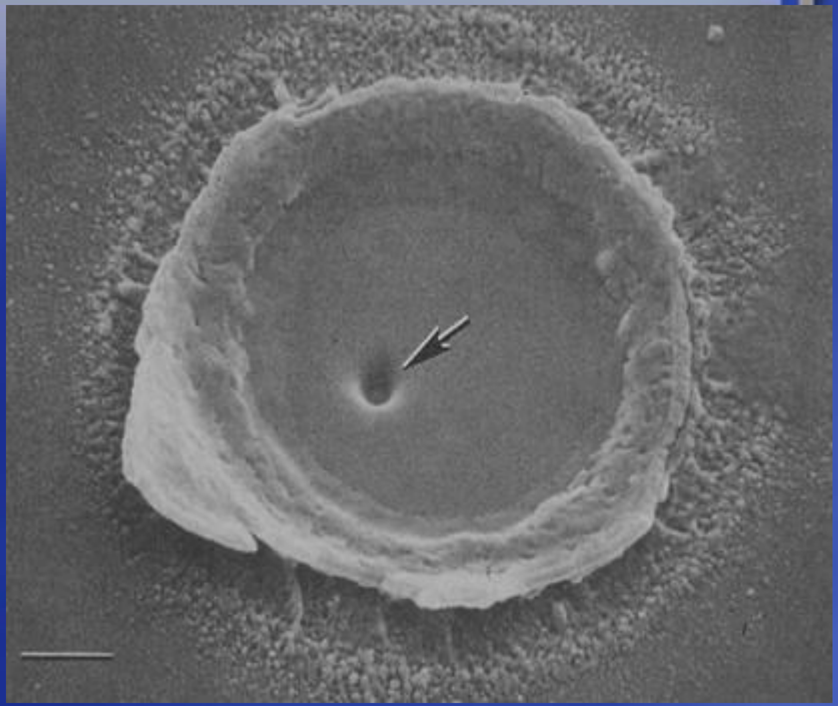
Effect on appressoria formation and penetration

1. Secretion anthranilic acid and transformation to 2,3 dihydroxybenzoic acid.
2. Modulation of appressoria formation by wax
3. Inhibition fungal cutinase and the penetration of germinated appressoria

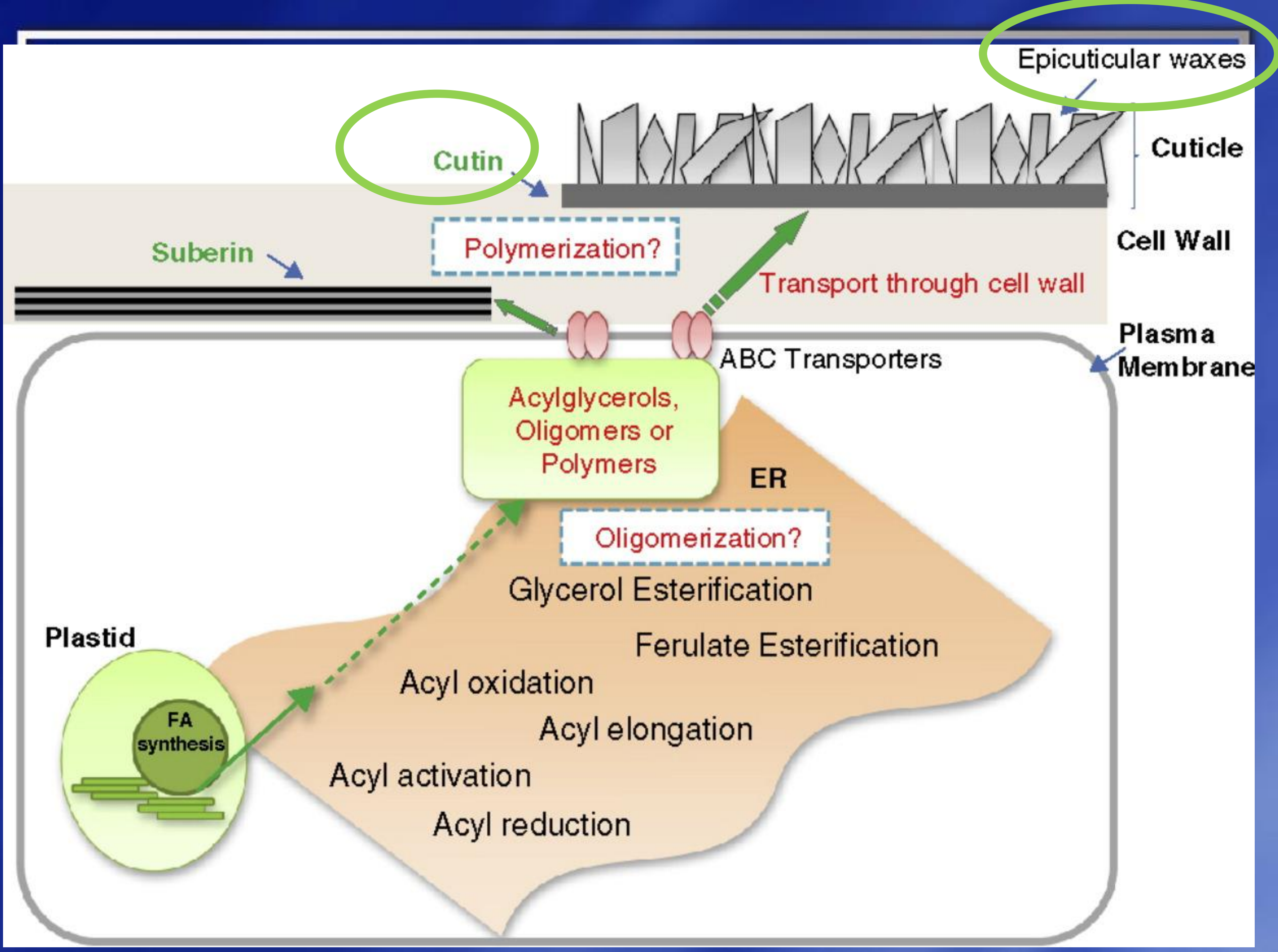
Appressoria penetration of the fruit cuticle and quiescence by Colletotrichum







Cutin is one of two waxy polymers that are the main components of the plant cuticle (epicuticular waxes+ cutin) which covers all aerial surfaces of plants


Epicuticular waxes
Cuticle
Cutin
Cell Wall
Suberin
Polymerization?
Transport through cell wall
Plasma Membrane
ABC Transporters
Acylglycerols, Oligomers or Polymers
ER
Oligomerization?
Glycerol Esterification
Ferulate Esterification
Plastid
Acyl oxidation
Acyl elongation
FA synthesis
Acyl activation
Acyl reduction

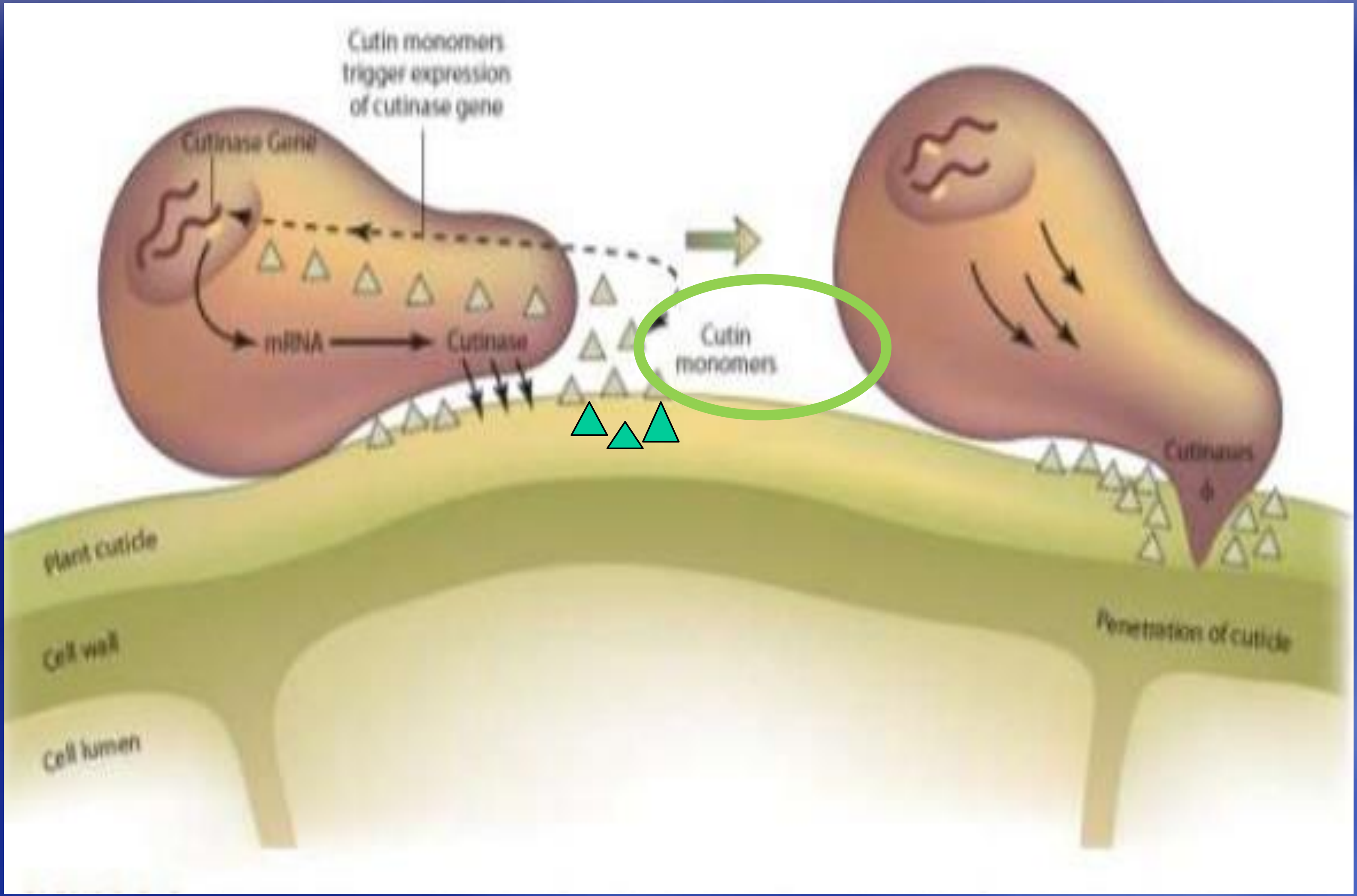
Fungal cutinase

Is an enzyme that catalyzes the chemical reaction

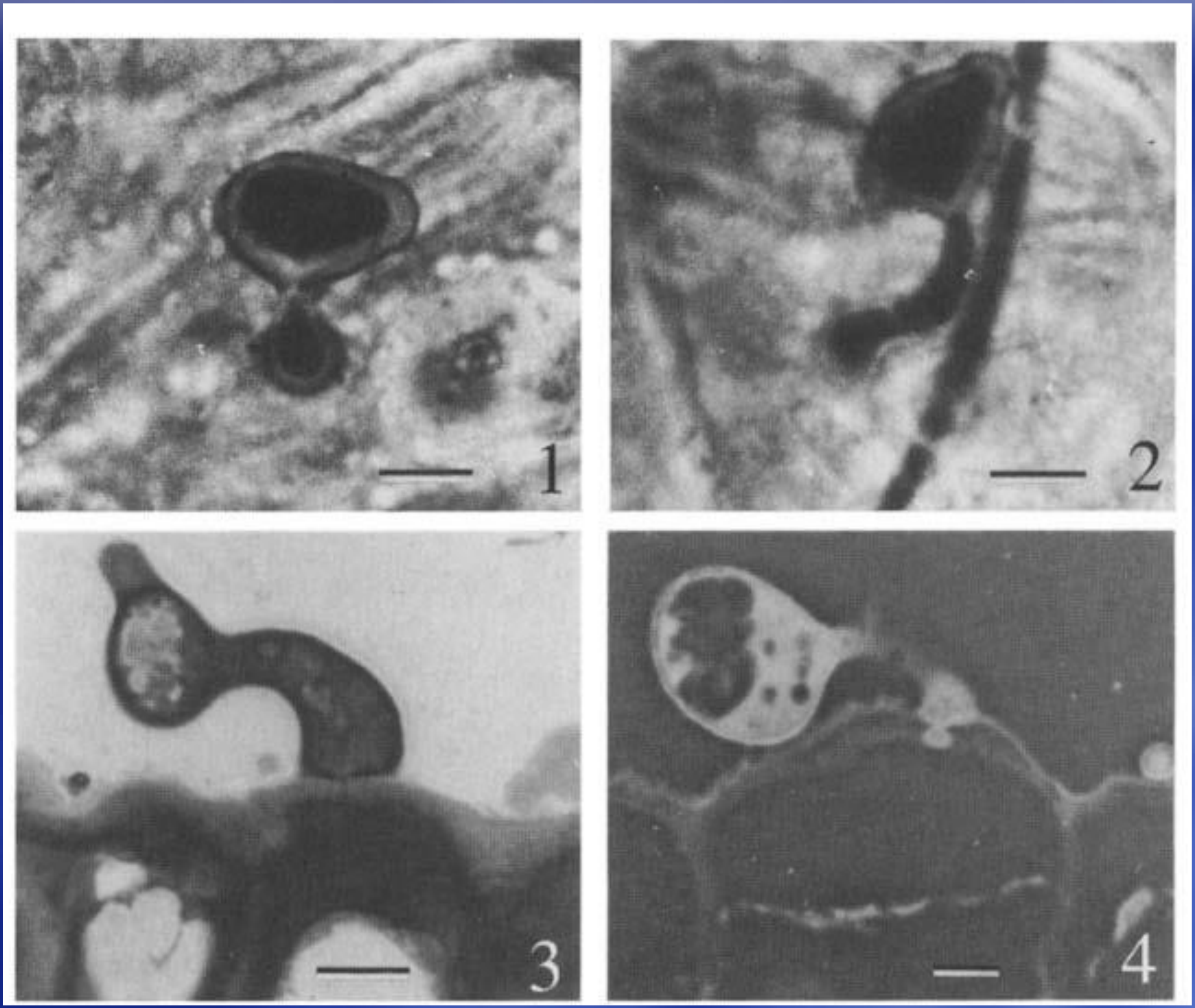
Plant cutin + H_2O → cutin monomers

Thus, enabling the fungal penetration of the hyphae through the cutin monomer products

This enzyme is a hydrolase,
specifically acting on carboxylic ester bonds and it is usually called as cutin hydrolase.


Cutin monomers
trigger expression
of cutinase gene
Cutinase Gene
mRNA
Cutinase
Cutin
monomers
Cutinases
Plant cuticle
Cell wall
Cell lumen
Penetration of cuticle

Appressoria of Monilinia in peach fruit



Fungal cutinase

Is an enzyme that catalyzes the chemical reaction

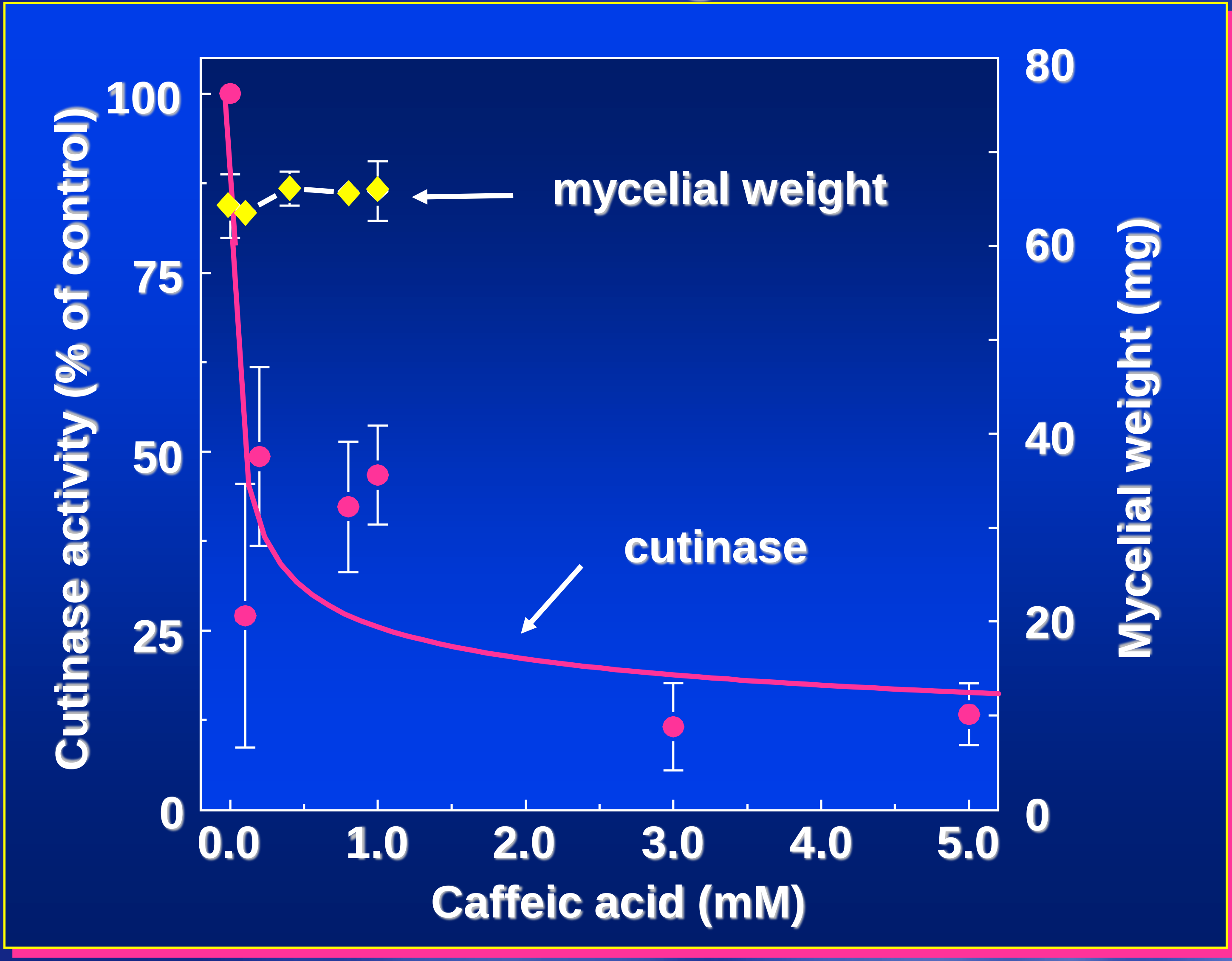
Plant cutin + H_2O → cutin monomers

Thus, enabling the fungal penetration of the hyphae through the cutin monomer products

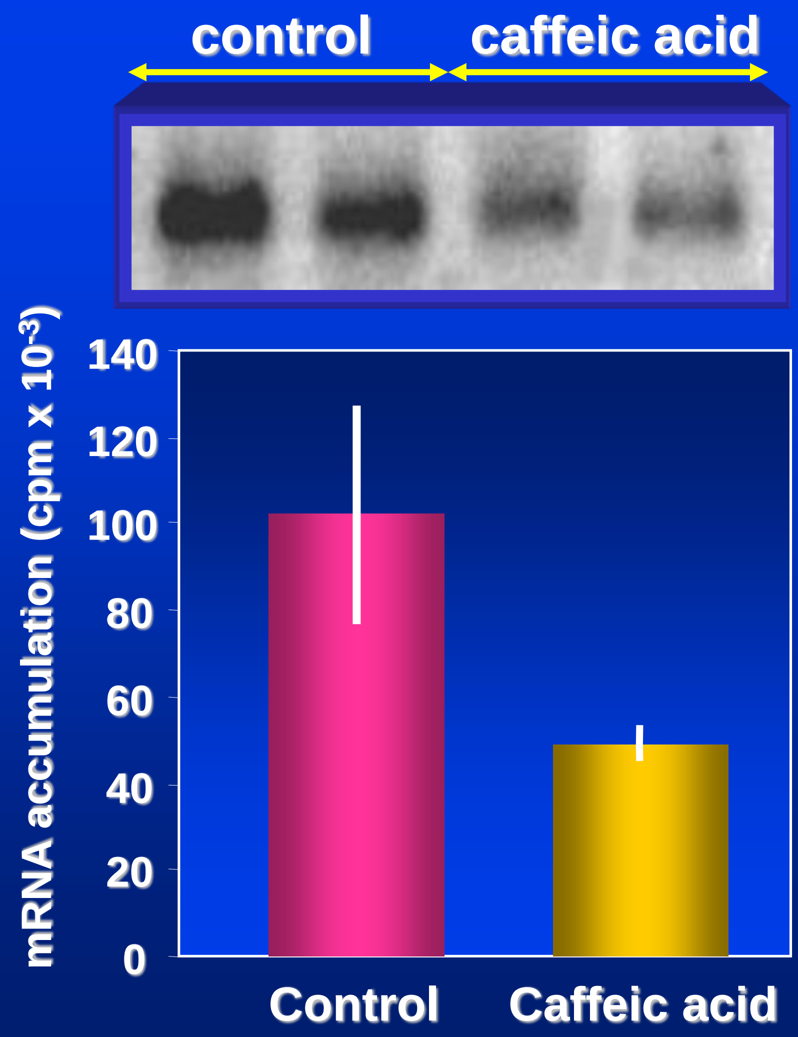
This enzyme is a hydrolase, specifically acting on carboxylic ester bonds and it is usually called as cutin hydrolase.

Is it possible that phenols present in the peel of the fruit may inhibit the activity of those enzymes?

Effect of fruit caffeic acid present in the peel of fruit peaches on the *Monilinia* cutinase and growth



Monilinia cutinase mRNA accumulation

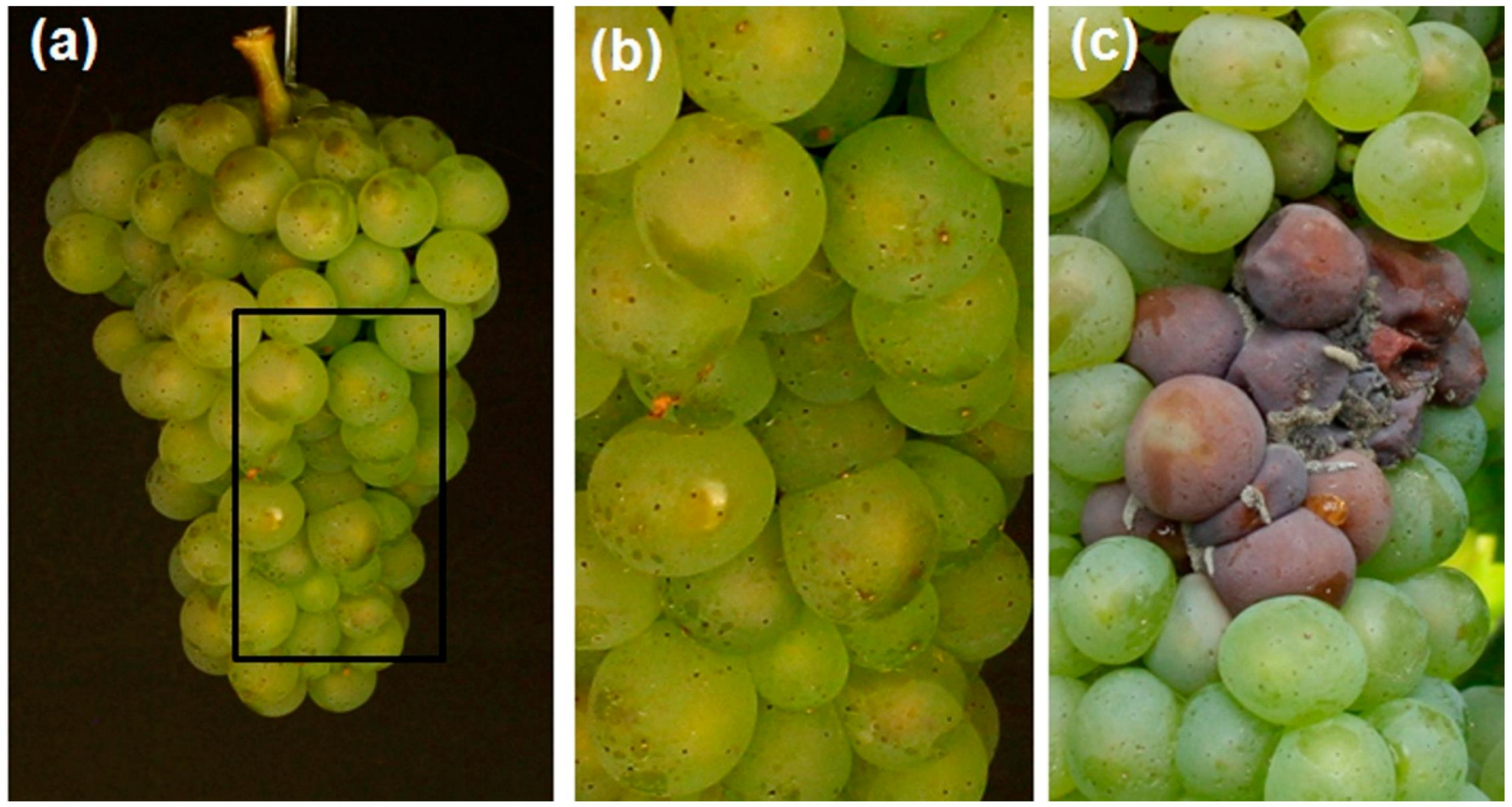


Host responses induced to direct penetration pathogens

Effect on appressoria formation and penetration

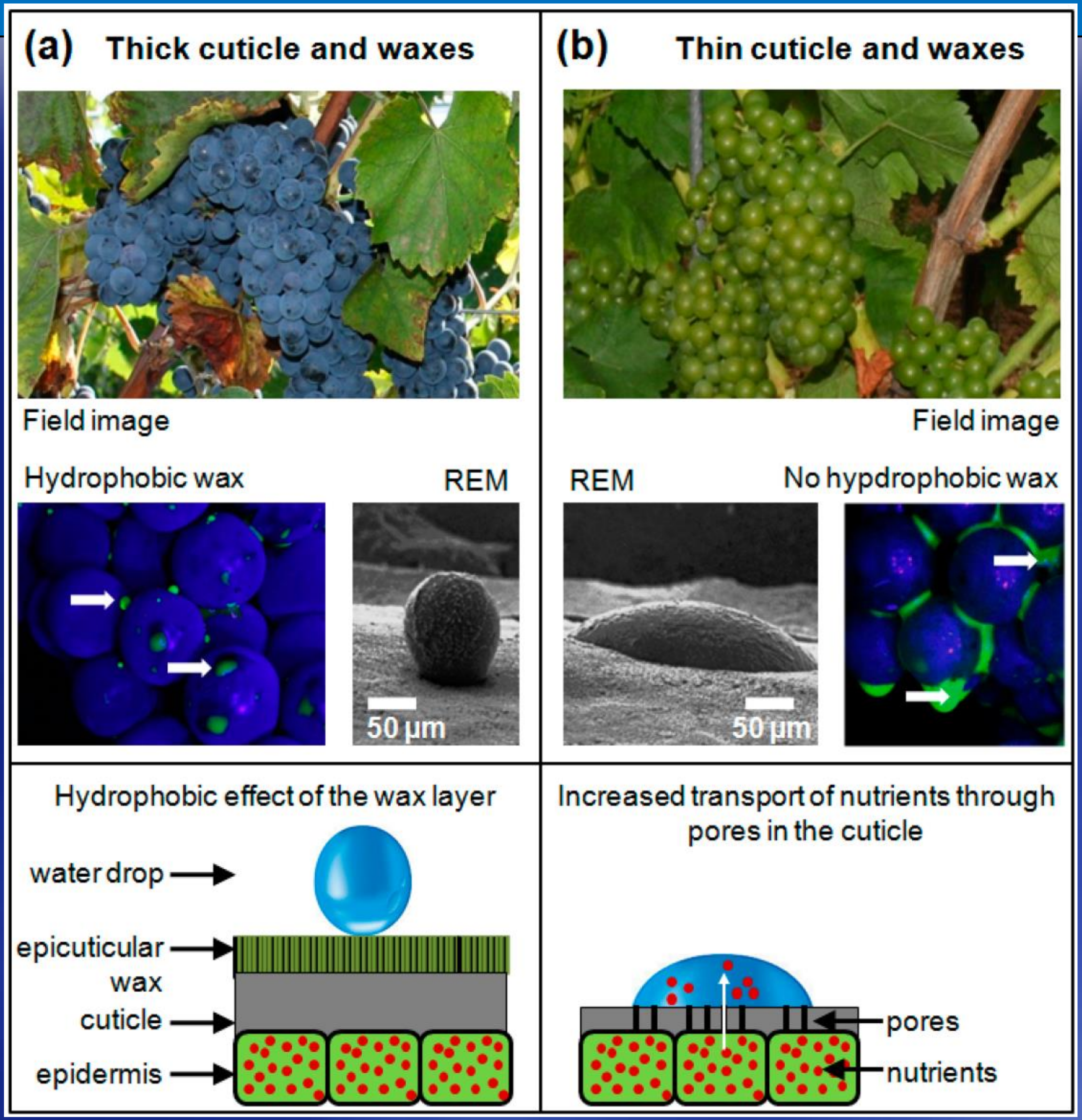
1. **Secretion anthranilic acid and transformation to 2,3 dihydroxybenzoic acid.**
2. **Modulation of appressoria formation by wax**
3. **Inhibition of penetration of germinated appressoria by inhibition of cutinase**
4. **Cuticle thickens**

Compactness of the bunches in Riesling cultivar



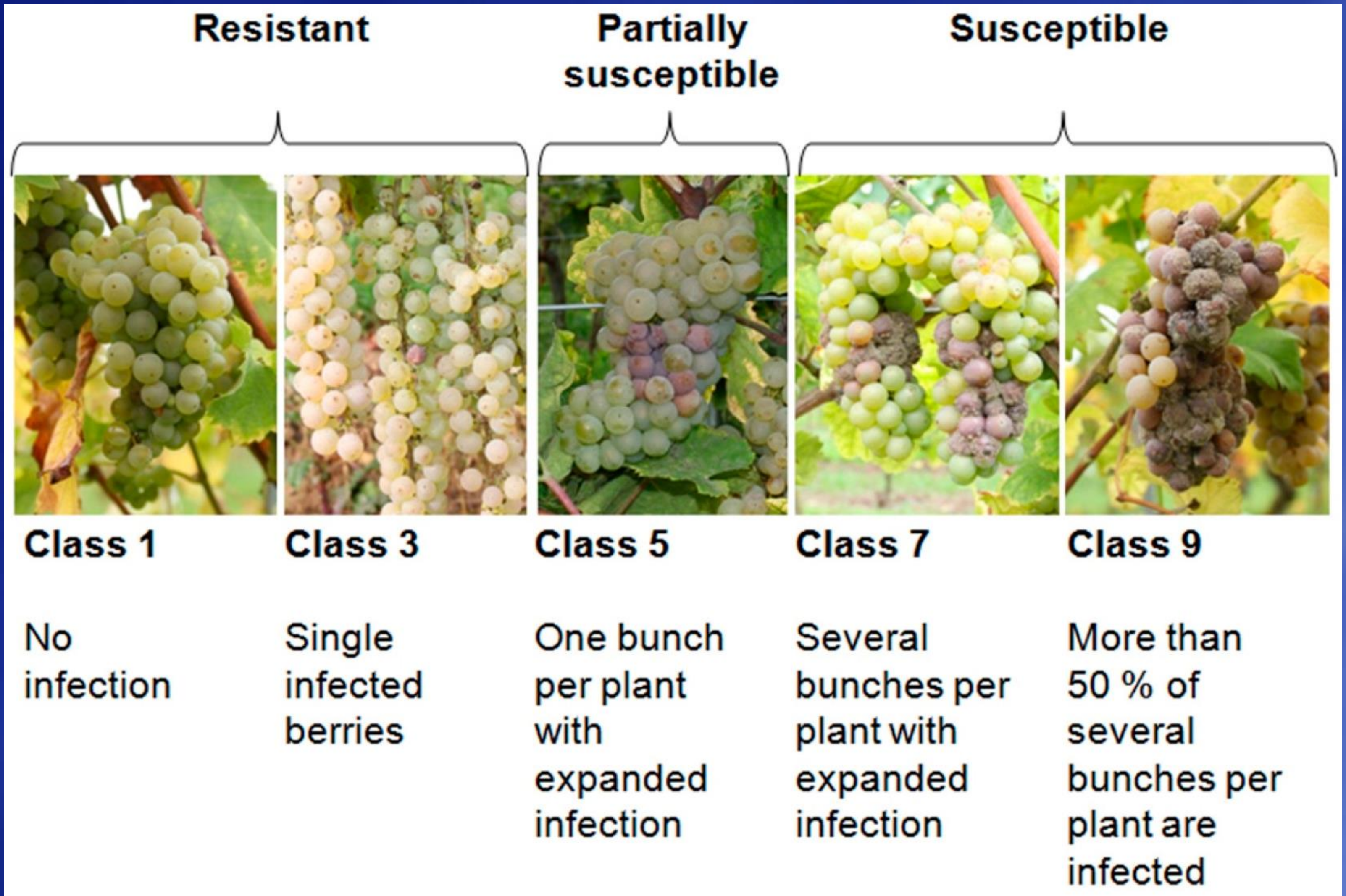
Impedance of the Grape Berry Cuticle as a Novel Phenotypic Trait to Estimate Resistance to *Botrytis cinerea.* Herzog et al. 2015 Sensors.

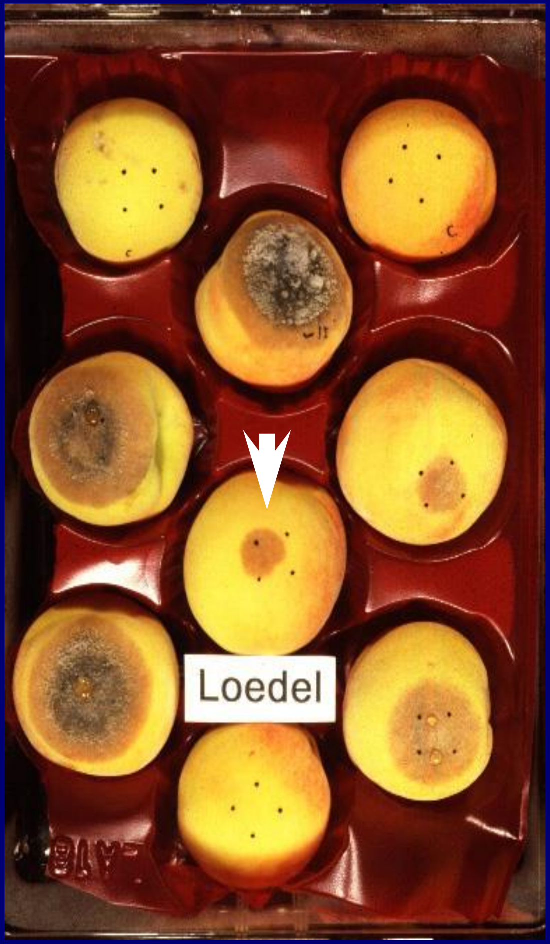
Function of the cuticle and epicuticular waxes as physical barriers.



(a) Grape berries with a thick cuticle and waxes, accessions Seibel 182 from the genetic repository in Siebeldingen was used. The hydrophobic characteristic of epicuticular waxes (wax layer) permits fast drying of berry surfaces; (b) Berries with thin cuticle and waxes cultivar 'Morio Muskat' is shown. Fluorescein (yellow-green) stained water

Classification of grapevine susceptibility to *B. cinerea* infection





Loedel is a susceptible commercial cultivar. 92,10-138 is an advanced experimental selection with brown rot resistant Bolinha heritage developed in UCDavis (CA)

Summary: Host responses induced to direct penetration pathogens

Effect on appressoria formation and penetration

1. Secretion anthranilic acid and transformation to 2,3 dihydroxybenzoic acid.
2. Modulation of appressoria formation by wax
3. Inhibition of penetration of germinated appressoria by inhibition of cutinase
4. Cuticle thickens

Summary

Host may modulated intial stages of fungal development dependent on the way of penentration of the pathogen

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<u>In wound penetrating pathogens</u>

Release of volatiles that affect fungal penetration

<u>In direct pepentrating pathogens,</u>

1. **Secretion anthranilic acid and transformation by the pathogen to 2,3 dihydroxybenzoic acid.**
2. **Inhbition of appressoria formation by wax**
3. **Inhibition of fungal cutinases produced by germinated appressorias**
4. **Cuticle thickens**