Pseudomonas savastanoi triggers the Hypersensitive Response of *Olea europaea* var. Galega Vulgar suspension cell cultures

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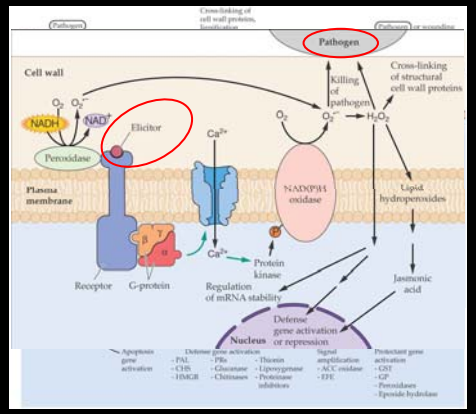
The Hypersensitive Response



(Buchanan *et al.*, 2000)

Olea europaea L.

Significant importance in Portuguese economy

- 340,000 acres of olive orchards
- 40,000 tons of olive oil / year
- 40% destined for exportation

Pseudomonas savastanoi

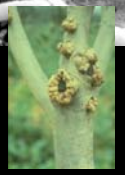
(Pseudomonas syringae pv. *savastanoi)*

Responsible for the olive knot disease

- Originates galls in the stems, shoots and leaves
- Drastically reduces productivy and quality of fruits

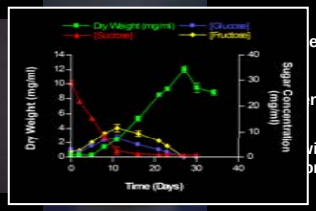
Enters the plant through wounds, leaf scars or bark cracks made by freezing

Spread by wind and rain



Establishment of an *in vitro* elicitation system

Establishment of suspension cell cultures of *Olea europaea* var. Galega Vulgar



Development of the elicitation system:

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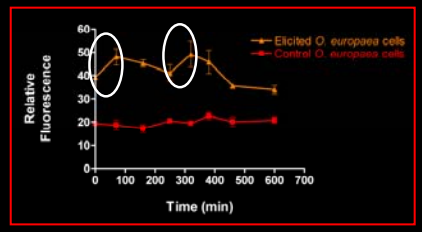
ROS production

Spectrofluorimetric quantification using the fluorescent probe H_2DCFDA



Two bursts of ROS production

50 min

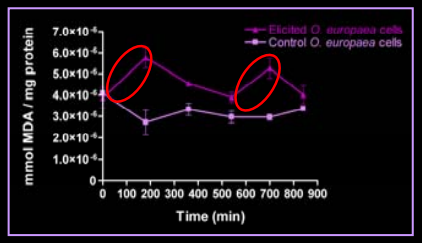
300 min

Pattern characteristic of the Oxidative Burst of incompatible interactions

Lipid peroxidation

Assessment of the amount of oxidative injury to the cells

MDA quantification using the TBA test



Two peaks in lipid peroxidation levels

100 min

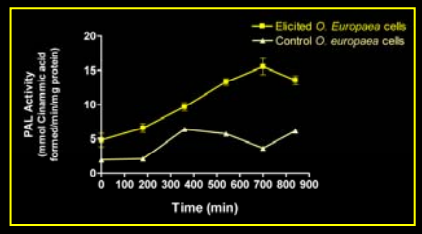
600 min

Peaks occur immediately after ROS production bursts

PAL Activity

First enzyme of the phenylpropanoid pathway

Spectrophotometrical quantification of trans-cinammic acid formation



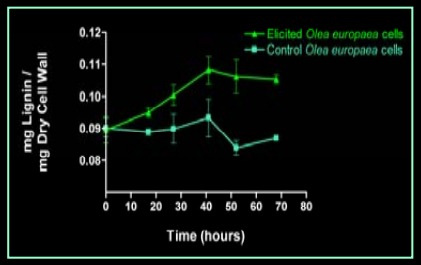
Significant increase in PAL activity in challenged cells

Indicates activation of defense mechanisms

Lignin Content

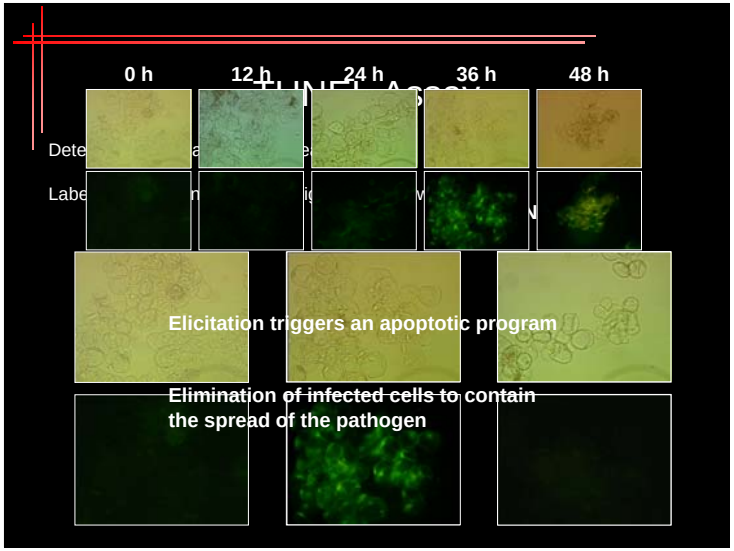
Main component of the cell wall

End product of the phenylpropanoid pathway



Elicited cells show higher lignin content in the cell wall

Reinforcement of the cell boundaries as a resistance process



Elicitation triggers an apoptotic program

Elimination of infected cells to contain the spread of the pathogen

Conclusions

The interaction between the variety Galega Vulgar of *Olea europaea* and the bacteria *Pseudomonas savastanoi* is apparently incompatible

- **Increased levels of ROS production**
- **Production of secondary metabolites essential to the resistance process**
- **Reinforcement of the cell boundaries**
- **Triggering of programmed cell death**

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