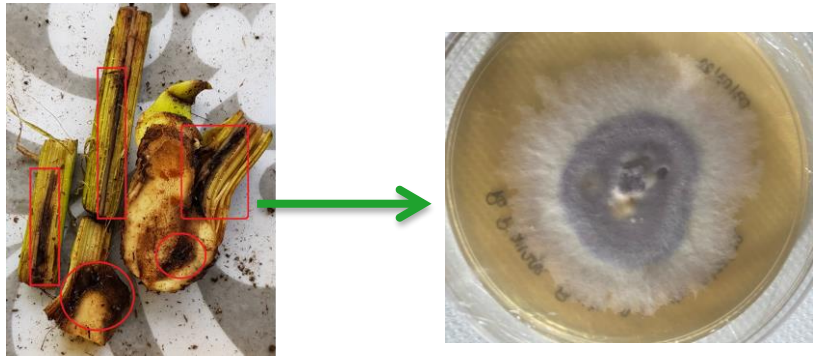
# Systemic resistances of banana as a potential tool to control *Fusarium oxysporum f. sp. cubense race 1*

*(Preliminary exp.)*



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# Partners

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Ecological functioning and sustainable management of banana and pineapple agrosystems
*(pest & disease management through rotation systems and systemic resistances)*

Centro Bioplantas, Universidad de Ciego de Avila, Cuba,

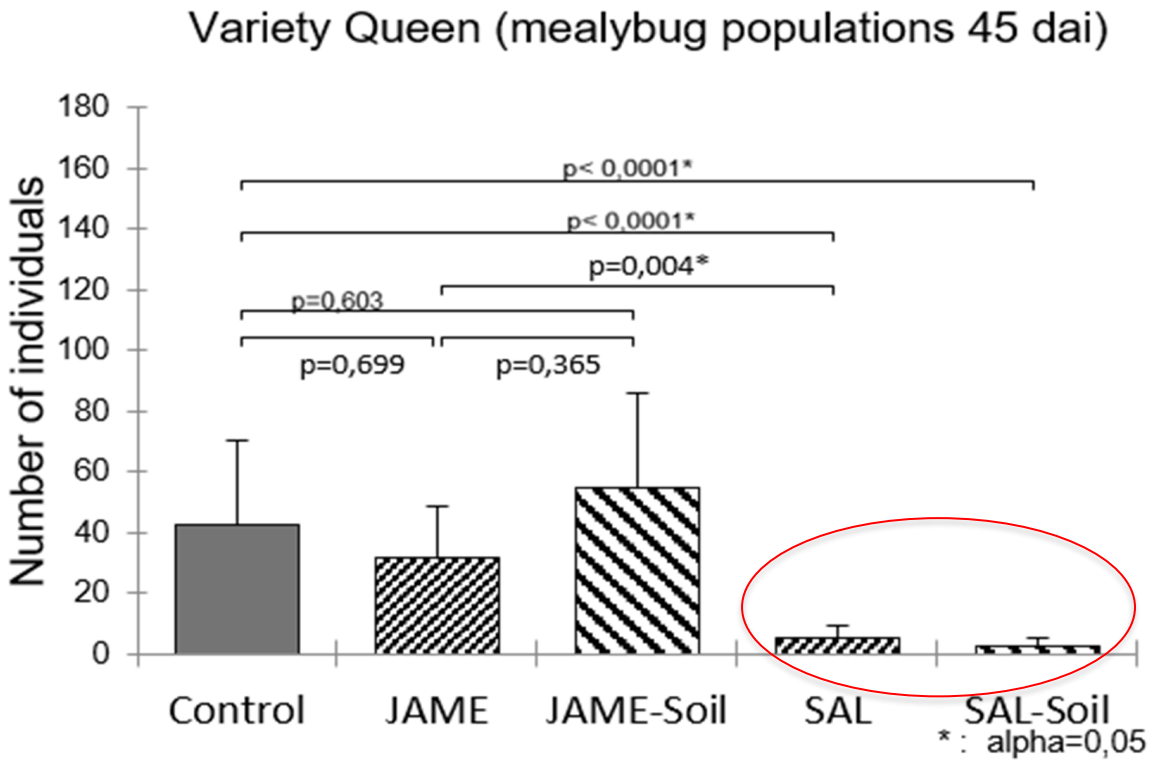
Laboratorio de Interacción Planta -Microorganismo, Centro de Bioplantas,
(Interaction Foc – banana, Phytophthora – pineapple, systemic resistances)

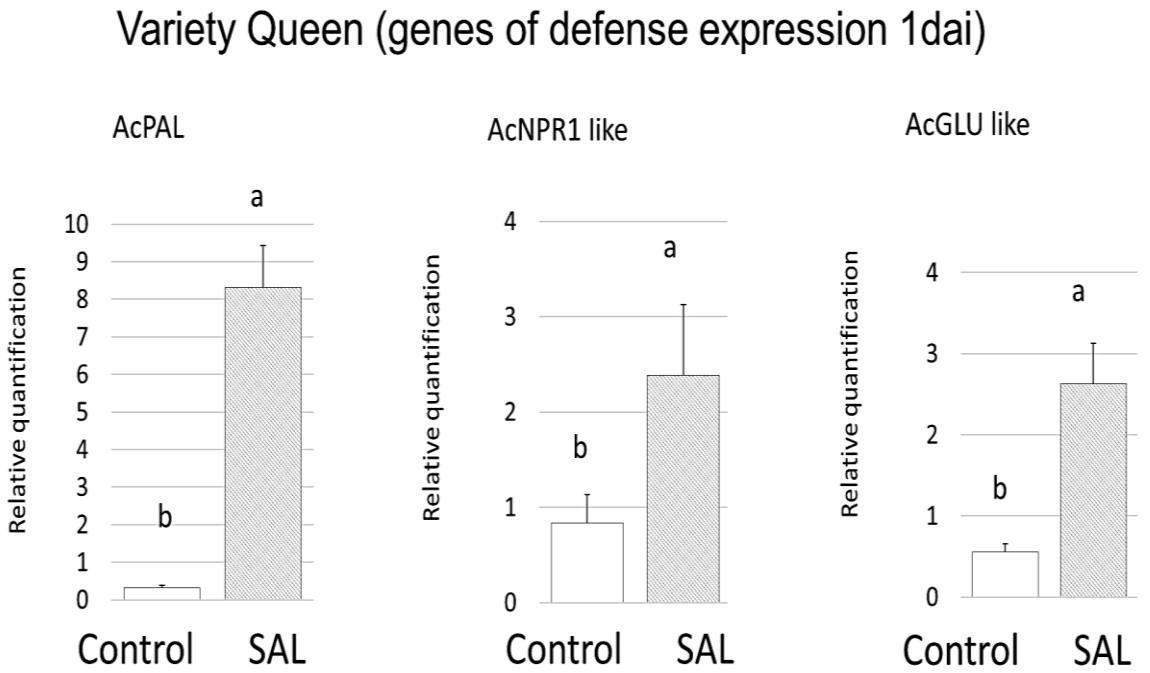
*Funding European : FEDER in Réunion and Erasmus+ (2022-2023)*

# Systemic Resistances = Inducible natural defenses

*Why Systemic Resistances to control Foc r1 ?*

*Former experiences in controling Mealybugs (wilt) in pineapple*

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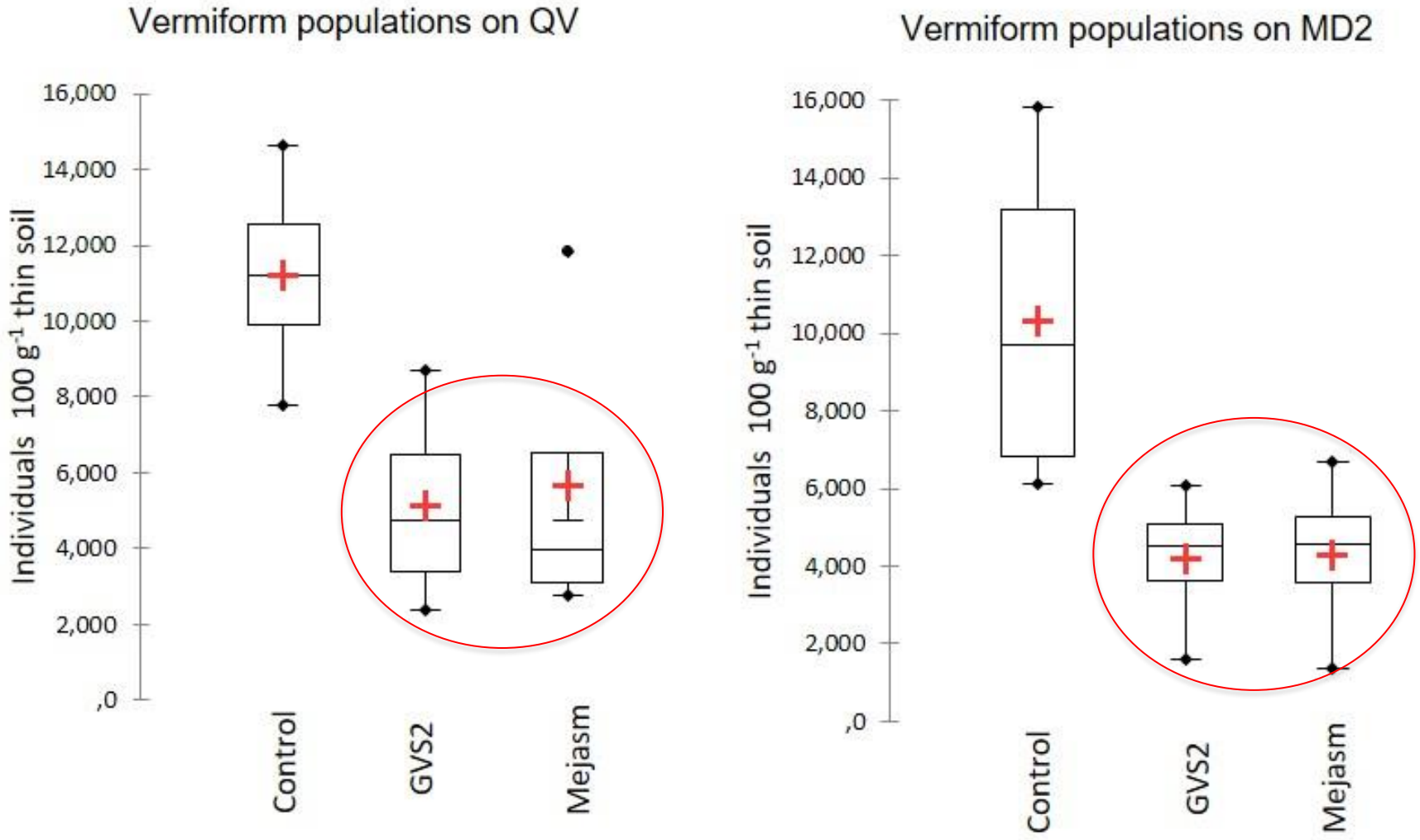
# *Former experiences in controling nematodes in pineapple (field level, Soler et 2020)*




*Systemic resistances to control nematodes with bacterial or chemical induction (methyl jasmonate, 0,1mM)*

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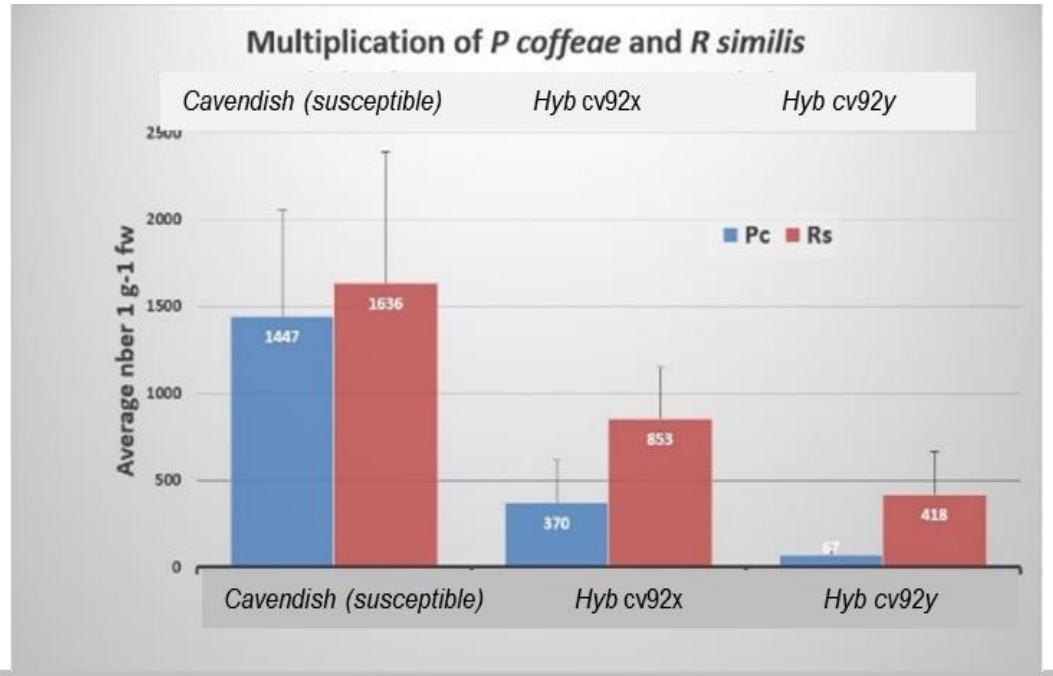
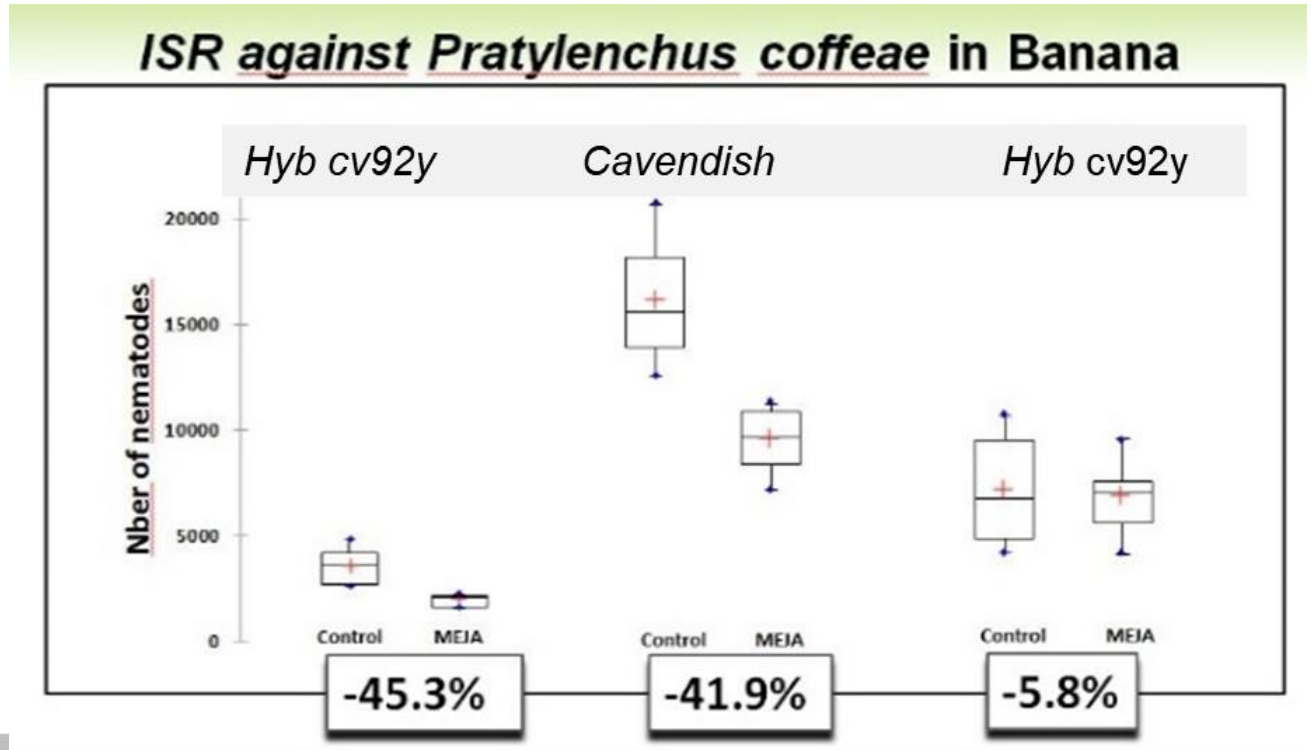
# *Former experience in controling nematodes in banana*




Estimates of the relative natural susceptibility to *P coffeae* & *R similis* of Cavendish (susceptible) of different banana varieties




ISR Stimulation by MEJA ($10^{-4}$M) to enhance tolerance to nematodes of Cavendish, CIRAD hybrid.

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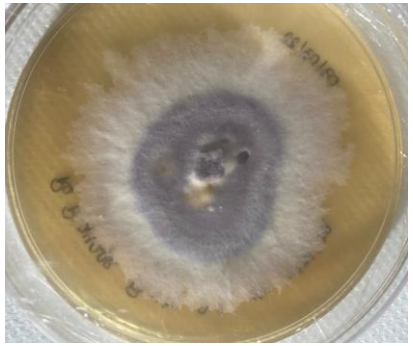
# Mat.Meth.

## *Fusarium oxysporum sp cubense r1*

*Field isolates tested on vitroplants Frayssinette and Manzano then re-isolated.*

*The inocula were produced from monosporic cultures of Foc r1*



Frayssinette (AA) and Manzano (AAB) are varieties susceptible to Foc r1

Foc r1 described as hemi-biotrophic fungus

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# *Systemic resistances : biological and molecular effects on different banana varieties*

**Biological effect** (symptoms)

Manzano and Frayssinette varieties, susceptible to Foc r1

- Acibenzolar S-methyl (Actigard®) (SAR)
- Jasmonates (Biojas®) (ISR)
- Salicylic ac & Methyl Salicylate (SAR)

(0.1mM and 1mM, 10ml/plant)

3 applications at 3 days interval, (inoculation 3 days later)

**Short term molecular effect**

Frayssinette (AA), Cavendish (AAA), Cirad Hybrid (AAA)

- Salicylic acid ) – SAR (0.1mM, 10ml /plant
- Methyl-salicylate – SAR (0.1mM, 10ml /plant )

1 application (inoculation 12h after treatment in susceptible var. and 48h in resistant var.)

Frayssinette (AA) and Manzano (AAB): susceptible to Foc r1
Cavendish (AAA) is tolerant to Foc r1 but susceptible to TR4
Cirad Hybrid (AAA): resistant to Foc r1 & TR4

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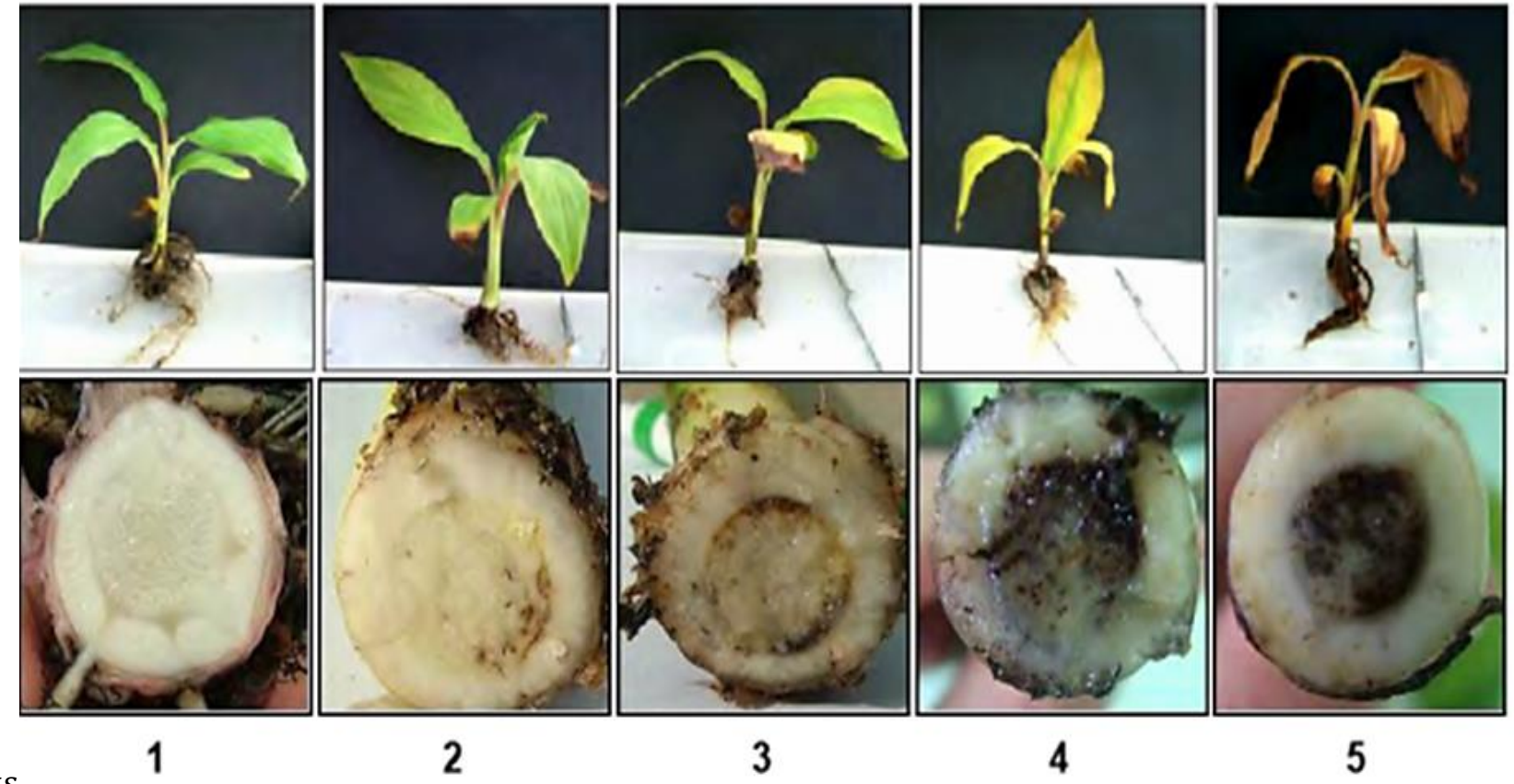
## Biological effect evaluation

*Symptoms evaluation : Scale of severity (S), (%) and incidence (I), (%)*

- External symptoms
- Internal symptoms




$$I\% = \frac{Number\ of\ wilted\ plants}{Total\ number\ of\ plants} \times 100$$

Sherwood & Hagedorn,1958

$$S\% = \frac{\Sigma(Number\ of\ wilted\ plants\ in\ a\ specific\ scale\ level\ x\ specific\ scale\ level)}{Total\ number\ of\ plants\ x\ maximum\ specific\ scale\ level} \times 100$$

# Mat.Meth.

## *Molecular effect evaluation*

| Molecular effect | Varieties | Timing |
|---|---|---|
| Short term modulation of expression of molecular markers after Foc r1 inoculation | - Frayssinette susceptible to Foc r1<br>- Cavendish tolerant to Foc r1, but susceptible to TR4<br>- Cirad Hybrid resistant to Foc r1 & TR4 | 12h after inoc.<br>48h after inoc.<br><br>48h after inoc. |

*- RNA extraction with the Qiagen Plant mini Kit on 150mg deep frozen root in liq $N_2$ (6 replicates).*

*(In house modification of Qiagen protocol for banana to protect RNA from latex and phenolic compounds)*

*- RTqPCR with Fast SybrGreen Mix on StepOne equipment (Applied Biosystem)*

***Molecular Markers tested :***

***- PAL, ICS, and NPR1 related to control of salicylic acid biosynthesis and SAR signaling pathway***

***- PR3, PR1 and CYS, related to defenses***

*Results normalized to Ctrl(-) (unstimulated – not inoculated controls),*
*and the graphic shows differences between Ctrl(+) and tests for each variety.*

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# Results

*Foc r1 and external symptoms on stimulated plants (SAR and ISR)*

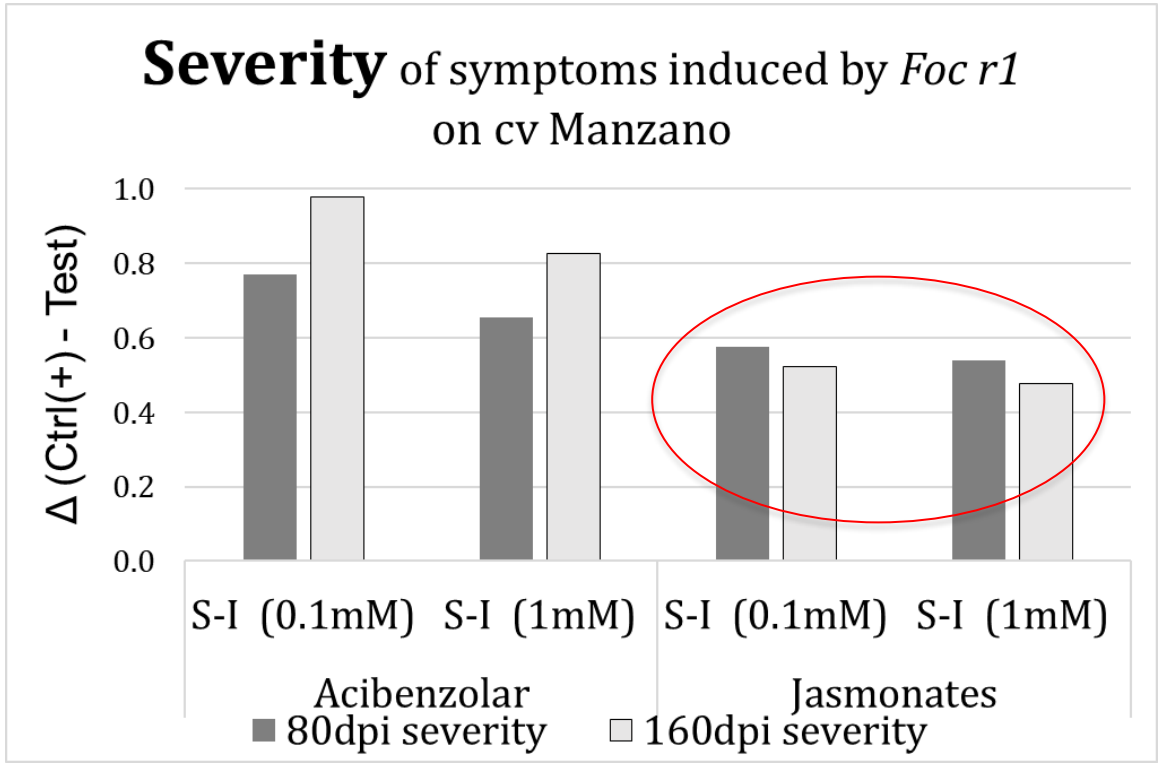
Variety Manzano

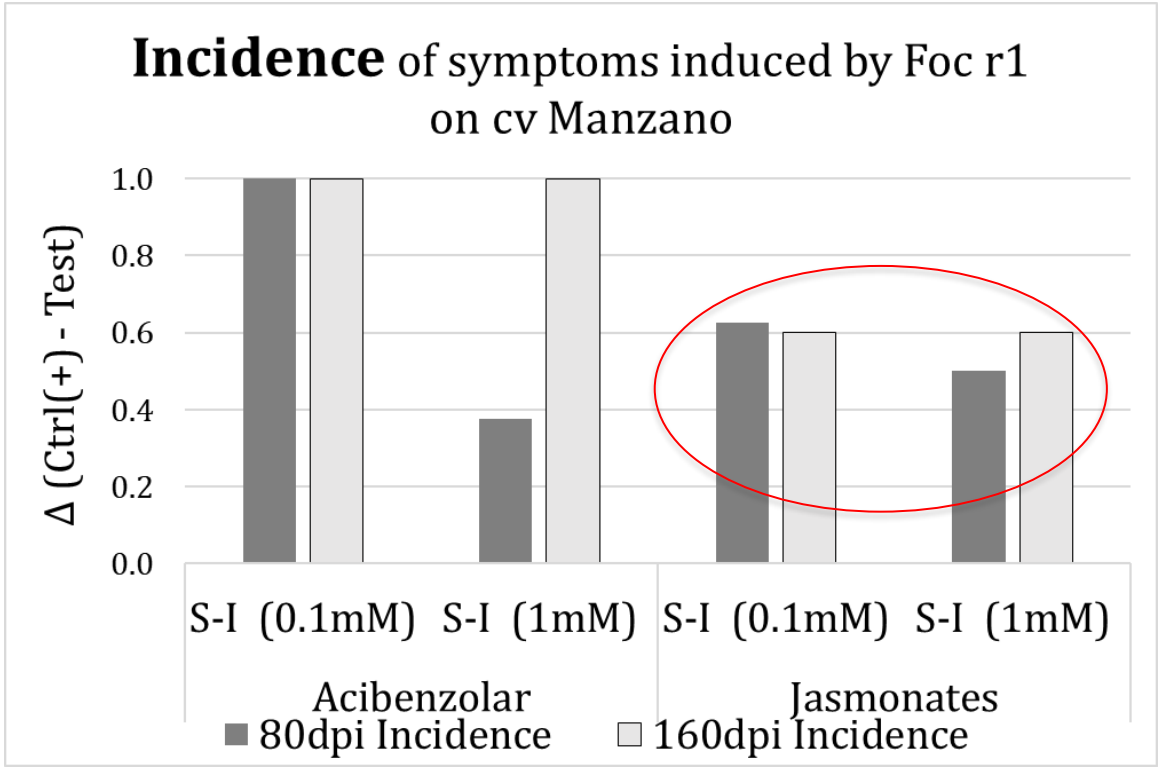
80dpi


160dpi

# Results

## *Foc r1 and external symptoms on stimulated plants (SAR and ISR)*




*Results normalized to Ctrl (+), graphic shows Ctrl (+) – test (unstimulated,Inoculated plants) – (stimulated and inoculated plants) (Anova)Tukey test, p≤ 0,05*

- ISR induced by **jasmonates** (0.1mM and 1mM) reduced significantly severity and incidence of symptoms at 80 and 160 dpi.
- SAR induced by **acibenzolar S-methyl** (1mM) reduced less severity of symptoms than jasmonates (+ Phytotoxicity ???)

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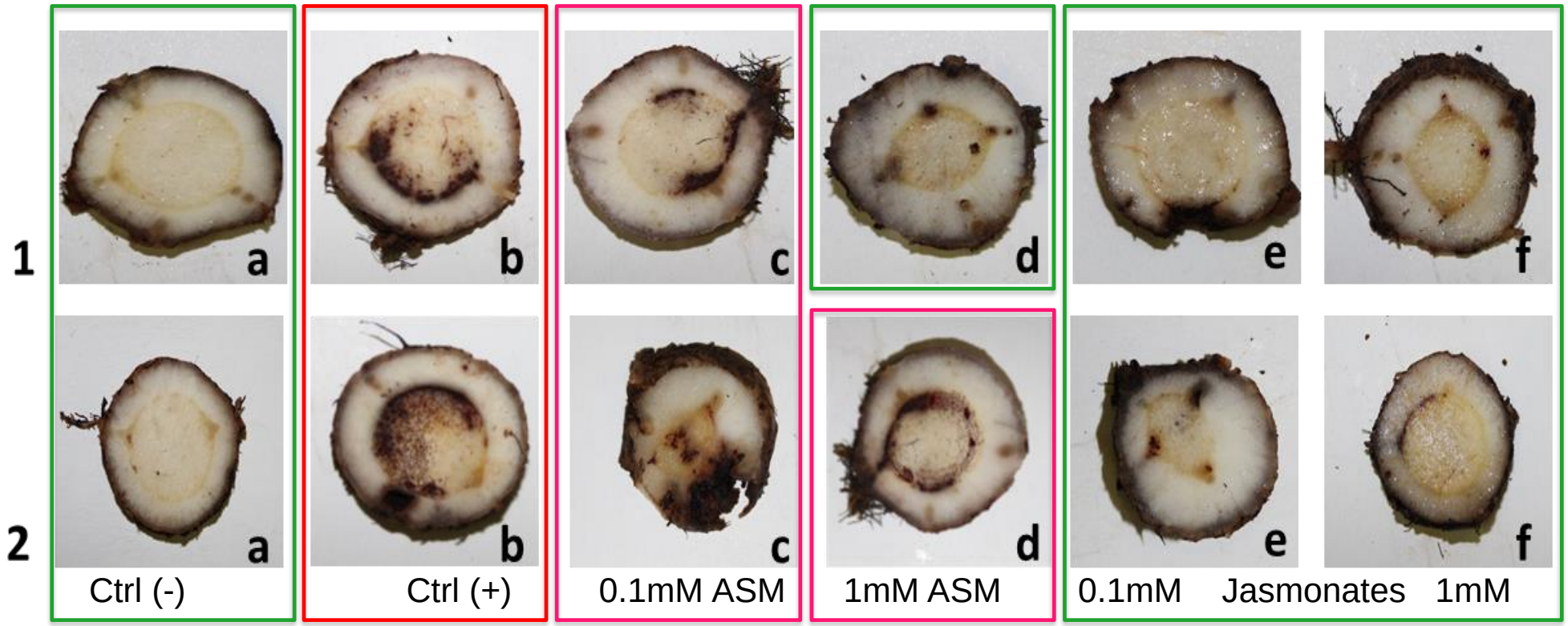
# Results

## *Foc r1 on internal symptoms on stimulated plants (SAR and ISR)*




- ISR induced by jasmonates (0.1 and 1mM) reduced internal symptoms at 80 and 160 dpi.
- SAR induced by Acibenzolar S-methyl (1mM) reduced slightly internal symptoms at 80 dpi.

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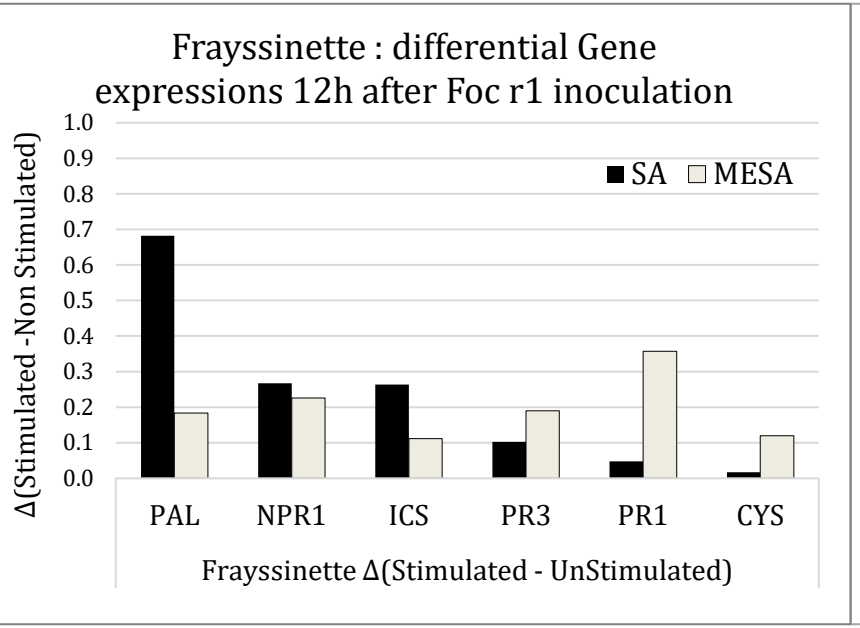
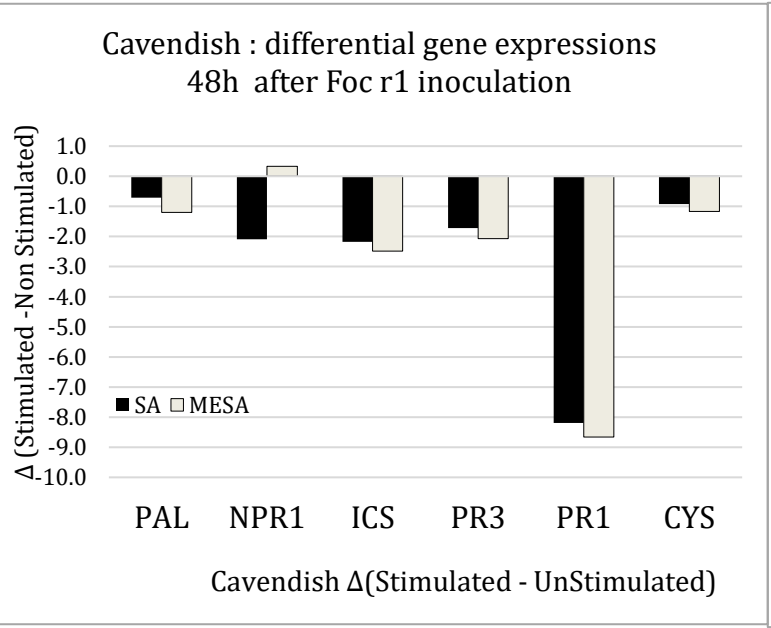
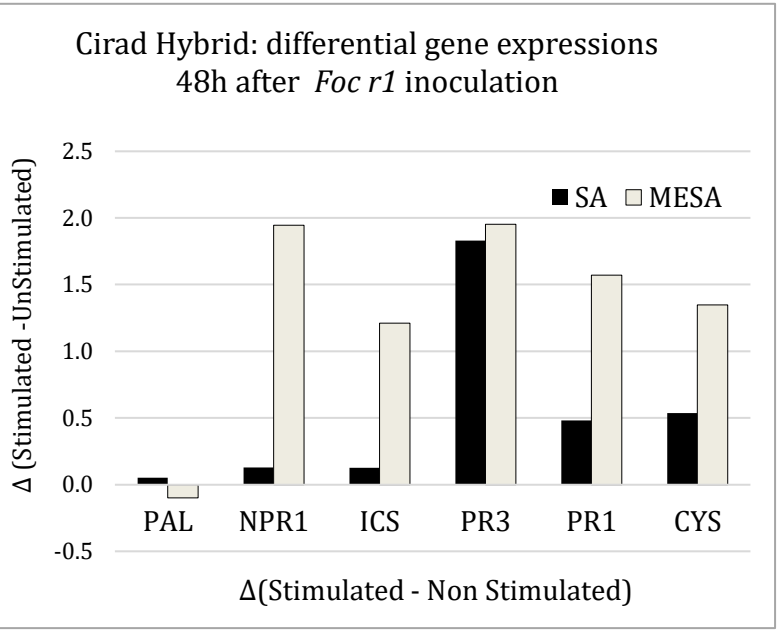
# Results

## *Foc r1 on molecular markers on stimulated plants (SAR)*




Hypothesis 1 : Foc r1 is biotrophic at early stage of infection (SAR), then necrotrophic later (ISR)

Hypothesis 2 : Foc r1 penetration is faster in sensitive varieties so timing for analysis
- 12hpi for sensitive var.
- 48hpi for resistant var.

- In Frayssinette : up-regulation of SAR pathway and SA synthesis (/SA), Defense proteins (/MESA)
- In Hybrid : up-regulation all markers except PAL (/MESA & /SA)

- In Cavendish : at 48h Ctrl(+) showed higher gene expression than stimulated plants,
(incorrect evaluation may result from time-shifted after stimulation and transient gene expression, need for a time-course expression of genes)

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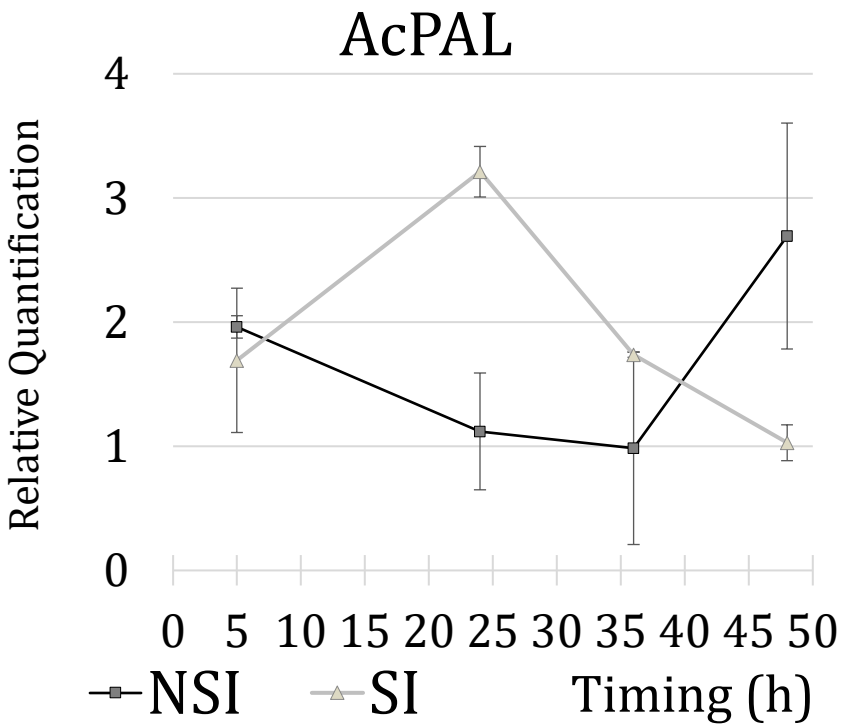
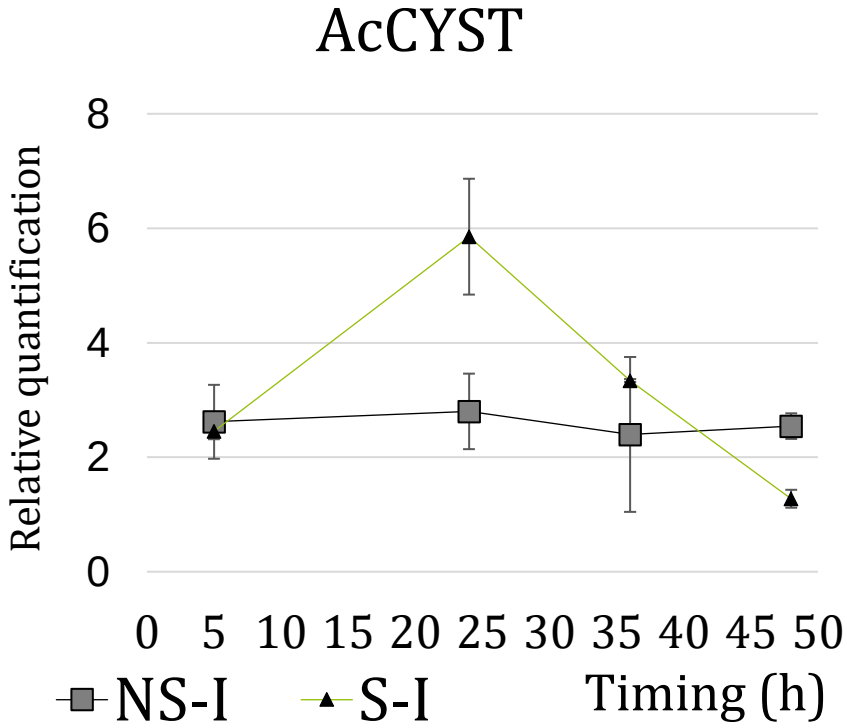
# Results

*Time-course for expression of molecular markers on stimulated plants and unstimulated plants (SAR)*




The time-course of genes expression in pineapple (short term effect) in the interaction mealybugs / pineapple.
An incorrect evaluation may result from the time-shifted after stimulation and transient expression of molecular markers.

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# Conclusion

*Foc r1 on primed plants (ISR or SAR )*

- ISR Priming inducers by jasmonates reinforced self-protection the plant. Reducing severity and incidence of wilt symptoms at very low concentrations (0.1mM) .

- SAR Priming inducers as salicylic ac and methyl-salicylate regulated genes expression in signaling pathway and defense genes in the different varieties, resistant or not , at early stages of *Foc r1* infection.

- The behavior of *Foc r1*, first biotrophic then necrotrophic, suggests a short-term effects for SAR during fungus penetration in roots, then a longer-term effect for ISR to limit the wilting of the banana.

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# Conclusion

*Foc r1 on primed plants (ISR or SAR )*

- Stimulation of Systemic resistances cannot be used as a simple pesticide

- An Optimal environment for systemic resistances is an ecological agrosystem that reduces stresses of the plants :
  - Reducing of pests inoculum
  - Then the primed plant takeover it self-defense

- Not all varieties respond positively to stimulation of systemic resistances.

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# *THANK YOU*
# *for your attention*

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