Systemic resistances of banana as a potential tool to control *Fusarium oxysporum f. sp. cubense race 1* (Preliminary exp.)

CIRAD, UPR GECO, F-97455 Saint-Pierre, Réunion, France. GECO, Univ Montpellier, CIRAD, Montpellier, France.

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)

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Systemic Resistances = Inducible natural defenses

**Why Systemic Resistances to control Foc r1?**

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

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**Variety Queen (mealybug populations 45 dai)**

<table>
<thead>
<tr>
<th></th>
<th>Number of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80</td>
</tr>
<tr>
<td>JAME</td>
<td>40</td>
</tr>
<tr>
<td>JAME-Soil</td>
<td>60</td>
</tr>
<tr>
<td>SAL</td>
<td>120</td>
</tr>
<tr>
<td>SAL-Soil</td>
<td>140</td>
</tr>
</tbody>
</table>

- p < 0.0001*
- p < 0.0001*
- p = 0.603
- p = 0.699
- p = 0.365

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**Variety Queen (genes of defense expression 1dai)**

<table>
<thead>
<tr>
<th></th>
<th>AcPAL</th>
<th>AcNPR1 like</th>
<th>AcGLU like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SAL</td>
<td>a</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Control</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SAL</td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

- a,b : Differents letters means significant differences at alpha = 0.05
Former experiences in controlling nematodes in pineapple (field level, Soler et al. 2020)

Systemic resistances to control nematodes with bacterial or chemical induction (methyl jasmonate, 0.1 mM)
Former experience in controlling 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^-4M) to enhance tolerance to nematodes of Cavendish, CIRAD hybrid.
Frayssinette (AA) and Manzano (AAB) are varieties susceptible to Foc r1.

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

The inocula were produced from monosporic cultures of Foc r1.

Foc r1 described as hemi-biotrophic fungus.
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
Symptoms evaluation: Scale of severity (S), (%) and incidence (I), (%)

- External symptoms

- Internal symptoms

\[
I\% = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100
\]

\[
S\% = \frac{\sum (\text{Number of wilted plants in a specific scale level} \times \text{specific scale level})}{\text{Total number of plants} \times \text{maximum specific scale level}} \times 100
\]

Sherwood & Hagedorn, 1958
**Molecular effect evaluation**

**Molecular effect**
- Short term modulation of expression of molecular markers after Foc r1 inoculation

**Varieties**
- Frayssinette susceptible to Foc r1
- Cavendish tolerant to Foc r1, but susceptible to TR4
- Cirad Hybrid resistant to Foc r1 & TR4

**Timing**
- 12h after inoc.
- 48h after inoc.

**Mat.Meth.**

- RNA extraction with the Qiagen Plant mini Kit on 150mg deep frozen root in liq N₂ (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.
Results

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

Variety Manzano

80dpi

160dpi

Ctrl (-) Ctrl (+) 0.1mM ASM 1mM 0.1mM Jasmnates 1mM
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 ????)
Results

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

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

- 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.
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 take over its self-defense

- Not all varieties respond positively to stimulation of systemic resistances.
THANK YOU for your attention