STUDY OF PLANT RESISTANCE INDUCERS (PRI) ON APPLE: FROM THE LAB TO THE FIELD

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Angers, France
Production in France

- 1.5 - 2 millions of tonnes
- 3rd European producer

37,300 cultivated ha
7,600 farms

Mean Nb of treatments / year = 35.1
(Mainly toward apple scab)

-30% in 11 years
+20% in 15 years

Source: Ecophyto R&D., 2009
Current situation regarding PRIs

In controlled conditions
Significant and reproducible control of disease

In the field
Control of disease rarely significant, with poor result reproducibility
Applicable to all crops

Global objective of the team: find answers for apple crop
Research on Plant Resistance Inducers

Applied research

Basic research

lab

Screening methods

Mode of action

Study of factors influencing effectiveness

Integration into pest management programs
Research on Plant Resistance Inducers

Applied research

- Lab
- Field

Basic research

- Mode of action

Screening methods

Study of factors influencing effectiveness

Integration into pest management programs
Screening of PRI candidates

PRI candidates

Defense activation

and/or

Protection efficacy

pathosystem?
Screening of PRI candidates on apple

PRI candidates

↓

Defense activation

↓

Protection efficacy

↓

qPFD

3 pathosystems

Apple scab
(Venturia inaequalis)

Fire blight
(Erwinia amylovora)

Rosy aphid
(Dysaphis plantaginea)
qPFD = Puce Faible Densité quantitative
(quantitative Low Density Array)

a ready-to-use molecular tool for the profiling of the expression of
28 defense genes by quantitative real-time PCR

1 plate = 1 cDNA sample

28 defense genes ★

SA ★

Antagonisme

JA ★

Additivité

ET ★

Barrières chimique & physique

Barrières chimique & physique

Barrières chimique & physique
Material and methods

Plant material

Apple seedlings
- population
- sustained production

Method

Pulv. of products at J0

Foliar disks prelevement at J0, J1, J2, J3

RNA extraction + RT

qPFD
Material and methods

Molecular analysis using the qPFD tool (RT-qPCR on 28 defense genes)

28 defense genes

- PR proteins
- Secondary metabolism
- Oxidative burst
- Cell-wall
- Signalling pathways

Calculation of a global rate of induced defense per product

Ranking of products against each other

Répression → Induction
Example of screening results

Screening of 25 commercial products

- Bion
- Trafos Mg-Ca-Si
- LBG01F34
- Kendal
- Armicarb
- PRM12
- Trainer
- Nectar Duo
- Optiplant petit pois
- Sucrose
- Chitoplant
- Fructose
- Alexin
- Régalis
- Defender
- H2O2
- Aliette
- Eau
- Vacciplant
- Sarriette
- Altex
- Fytosave
- Vitalgue
- BasFoliar SI
- Sérénade max
- Boralgine
- Isomag
- VG+
- Kendal cops

- Bion 50 WG  functionnal analog of SA (ASM)
- Trafos Mg-Ca-Si  P, Mg, Ca, Si
- LBG01F34  potassium phosphonate (KHP)
- Kendal  organic matter, glutathion
- Armicarb  potassium bicarbonate

- Market authorization as phytopharaceutical
- 4 synthetic products / 5
- Biocontrol product (NS 11/2016)
Research on Plant Resistance Inducers

Applied research

- lab
- field
- Screening methods
- Study of factors influencing effectiveness
- Integration into pest management programs

Basic research

- Mode of action
**Method**

Grafted genotypes

**Results**

Global rate of defenses

Genotypes (susceptibility to apple scab)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Abiotic stress</th>
<th>Interaction with other inputs</th>
<th>Persistence of action</th>
<th>Quality of the application</th>
<th>Durability of the induced resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elstar (+)</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fuji (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gala (++++)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Golden (++++)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pink Lady (+++++)</td>
<td></td>
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</table>
Protection test against apple scab

Genotype | Abiotic stress | Interaction with other inputs | Persistance of action | Quality of the application | Durability of the induced resistance

Results

Severity of apple scab
Hypothesis on genotypes exhibiting the same susceptibility level

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- No protection with the PRI
- Slight response to PRI
- Too low defense level

= constitutive level of defenses
≠ response to PRI

= = response to PRI
Mobilization of apple geneticists at Angers (C.E. Durel, IRHS)

Partial resistance (apple scab and fire blight) vs. Constitutive expression of defenses

Genotype | Abiotic stress | Interaction with other inputs | Persistence of action | Quality of the application | Durability of the induced resistance

PRI efficacy

400 génotypes

genetic determinism explaining the difference of PRI effectiveness?
Question

Initial $\phi$ state

PRI treatment

Elicited state

Genotype  | Abiotic stress | Interaction with other input | Persistence of action | Quality of the application | Durability of the induced resistance

Complexe experiment designs!
Example of experiment ($\Delta$Temp. day/night)

Result

Conditions to be diversified to answer the question!

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<table>
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<tr>
<th>Relative infection (%)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Bion</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
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</table>
Find a way to measure this initial $\phi$ state → Receptivity level to PRI
Measure of the receptivity level: 2 projects

1 - Expression of abiotic stress-responsive genes

Soon available!

2 – Transposable element activation (retrotransposons)

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Some results with fungicides, insecticides, thinning agents,…

dPFD analysis with inputs applied alone
- Soil fertilization (nitrogen)

PRI or water

≠ fertilization regimes

- Foliar fertilizers (oligoelements)

PRI or water ± foliar fertilizers

Defence analysis + protection tests

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**Tavinnov**
• **Soil fertilization (nitrogen)**

![Fire blight](image1.png)

![Graph showing incidence of fire blight](image2.png)

- N supply:
  - 7 days before PRI
  - 14 days before PRI

• **Foliar fertilizers (oligoelements)**

6 products tested in combination or applied 6 days before treatment: no identified interactions
Weekly applications of PRIs can be recommended in orchard, especially during the growing season.
Discrepancy between controlled conditions / Orchard

Orchard (Traditionnal sprayer)  Controlled-conditions (spray gun)

Purchase of an automated and adjustable spray chamber (2019)
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Mobilization of population geneticists of *Venturia inaequalis* (B. Le Cam, IRHS)

1) **Controlled conditions**

![Experimental evolution diagram]

- Inoculation 20 strains
- Elicited plants
- Control plants
- Comparison of strain aggressiveness
- SNP analysis

2) **Orchard**

**Sampling** of strains on trees that have received PRI-treatments for several consecutive years (100 strains)
Research on Plant Resistance Inducers

Applied research

Basic research

lab

Screening methods

Mode of action

field

Study of factors influencing effectiveness

Integration into pest management programs
National network for the field study

8 experimental sites in 3 production areas

Control for apple Scab (spring) and postharvest diseases (autumn)
Integration of PRI into pest management programs (toward apple scab)

**Strategy** consensual protocol between partners

- **Primary contamination**
  - March to July...
  - Classical IPM
  - Light IPM
  - Light IPM + PRI every week

- **Secondary contamination**
  - 15 days after the last lesions
  - Classical IPM

**Fungicide treatments managed with an enhanced acting threshold compared to the IPM (RIMpro apple scab prediction model)**

**Apple scab risk (RimPro values)**

IPM = Integrated Pest Management
Integration of PRI into pest management programs (toward apple scab)

Expected result
Integration of PRI into pest management programs (toward apple scab)

**Strategy in 2015**  
Light IPM = systematic **preventive** fungicides (any risk)  
**curative** fungicides if RIM value > 800 (severe risks)

**Results**

Results in most experimental units

- Too low acting threshold in light IPM
Integration of PRI into pest management programs (toward apple scab)

Strategy in 2016  Light IPM = no fungicide for the 1st risk  
  preventive and curative fungicides for severe risks

Results

40% of fungicides during the period (Nb treatment = 12)
But 8,5 PRI applications

In 2017: focus on 3 PRI to increase the number of experimental sites
Integration of PRI into pest managment programs (toward apple scab)

**Strategy in 2017**
Light IPM = no fungicide for the 1st risk preventive and curative fungicides for severe risks

New modalities = NT (no fungicides) + PRI

**Results**

- 50% of fungicides during the periode (Nb treatment = 12) With 8 PRI applications
- PRI effectiveness in fungicides-free orchard
- Non-reproducible results with some PRI
Integration of PRI into pest management programs (toward postharvest diseases)

**Strategy**  
Consensual protocol between partners

Application of PRI 6-8 weeks before harvest (depending on the rain)

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**Gloesporiose** (summary on 3 years)

<table>
<thead>
<tr>
<th></th>
<th>Infected fruits (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>a</td>
</tr>
<tr>
<td>Arnicarb</td>
<td>a</td>
</tr>
<tr>
<td>Bion</td>
<td>a</td>
</tr>
<tr>
<td>LBG</td>
<td>b</td>
</tr>
<tr>
<td>Trafos</td>
<td>b</td>
</tr>
<tr>
<td>Kendal</td>
<td>b</td>
</tr>
<tr>
<td>Ref</td>
<td>b</td>
</tr>
</tbody>
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Storage of the harvest in a cold room for a period of > 3 months
Integration of PRI into pest management programs

Unintended effect of PRI application?

Quantification of major allergenic proteins (PR5 and PR10) in fruits when PRI have been applied in spring or before harvest

Results (ELISA)

No impact of the PRI use on the fruit allergenicity
Results summary

Field effectiveness

LBG (K-phosphonate)

+++ 

Plant resistance induction, and antimicrobial activity?

Other products

+/-

Too good to be true!

Factors influencing effectiveness

Much work remains to be done!

Conidia germination

Fungal growth

Conidia germination (%)

400000
600000
800000
1000000
1200000
1400000

Fungal growth

Water

LBG

 factors influencing effectiveness
Outlooks on PRI

In controlled conditions

- Effect on the PRI efficacy:
  - genotype
  - fertilization
  - abiotic stress

- Durability of the induced resistance

In the field

- Protocol for integration of PRI:
  - PRI positioning

- Improvement of prediction models in taking into account:
  - the plant's receptivity to disease and PRI according to abiotic constraints

- Durability of the induced resistance

- Crossover with other alternatives?
  - resistant genotypes (partial)
  - physical technics (rain cover)
Registered PRI on apple (France)

4 PRI

- **Regalis®** (prohexadione-Ca) - BASF -
  (apple scab / fire blight / aphids / powdery mildew)

- **Vacciplant®** (laminarine) - Goëmar -
  (apple scab / fire blight)

- **Sérénade® Max** *(Bacillus subtilis)* - BAYER -
  (apple scab / fire blight)

- **Aliette® Flash** (fosetyl-Al) - BAYER -
  (fire blight)

1 PRI + fungicide

- **Delan® PRO** (Dithianon + K-phosphonate) - BASF -
  (apple scab)

1 PRI soon registered

- **Bion® 50WG** (Acibenzolar-S-methyl) - SYNGENTA -
  (apple scab / fire blight)