Contrasted spatio-temporal dynamics of fungicide resistance and its drivers in the pathogenic fungus *Zymoseptoria tritici* in France

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Introduction

Background

Pesticides efficacy loss due to resistance

« Stable, inheritable adjustment by a fungus to a fungicide, resulting in a less than normal sensitivity to that fungicide »

(EPPO, 1988)
Introduction

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Pesticides efficacy loss due to resistance

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Increased number of sprays or doses to keep to the same level of protection
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**Environnamental and human health issues**

*Ecophyto plan,
Agroecological project for France*

Pesticides efficacy loss due to **resistance**

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Increased number of sprays or doses to keep to the same level of protection

Purpose

Better management of resistance in agriculture

Need to increase our capacity to predict the dynamics of resistance

Retrospectively study the spatio-temporal evolution of resistance:

• Is the evolution of resistance homogeneous in France?
• Is the regional fungicide use a major driver of resistance selection?
Introduction – Biological model: *Zymoseptoria tritici*

Responsible for the **septoria tritici blotch (STB)**, studied here on **winter wheat**

- **Main pathogen on wheat**: ~18qt/ha -> 2Md€

(Fones & Gurr, 2015)
Introduction – Biological model: *Zymoseptoria tritici*

Responsible for the **septoria tritici blotch (STB)**, studied here on **winter wheat**

- **Main pathogen** on wheat: \(~-18qt/ha \rightarrow 2Md\€\)
- **Fungicides are the main control method**: 70% of European fungicides

(Fones & Gurr, 2015)
Introduction – Biological model: *Zymoseptoria tritici*, resistance status

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- Main pathogen on wheat: ~18qt/ha -> 2Md€
- Fungicides are the main control method: 70% of European fungicides
- Large diversity of fungicides: 5 chemical families

![Diagram showing the timeline of fungicide usage from 1970 to 2010 with different chemical families highlighted: Multisites, Benzimidazoles, DMI, QoI, SDHI.](image_url)
Introduction – Biological model: *Zymoseptoria tritici*, resistance status

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- **Main pathogen** on wheat: ~18qt/ha -> 2Md€
- **Fungicides** are the **main control method**: 70% of European fungicides
- Large **diversity** of fungicides: 5 chemical families
- **Diversity in resistances**: 3 mechanisms

**1970** | **1980** | **1990** | **2000** | **2010**
--- | --- | --- | --- | ---
**Multisites** | | | | |
**Benzimidazoles** | | | | |
**DMI** | | | | |
**QoI** | | | | |
**SDHI** | | | | |

« **All-or-nothing** »: **qualitative resistance**

« **Continuum** »: **quantitative resistance**
Introduction – Biological model: Zymoseptoria tritici, resistance status

Responsible for the Septoria tritici blotch (STB), studied here on winter wheat

- Main pathogen on wheat: ~18qt/ha -> 2Md€
- Fungicides are the main control method: 70% of European fungicides
- Large diversity of fungicides: 5 chemical families
- Diversity in resistances: 3 mechanisms
- Some resistances are well established in populations

« All-or-nothing »: qualitative resistance
« Continuum »: quantitative resistance
• **Resistance dynamics**

  -> control and treated plots, yield, year, wheat variety, sampling date, ...

  ![Map showing 1013 trials](image)

  (Garnault et al., 2019)
Materials – Databases per region

- Resistance dynamics


-> control and treated plots, yield, year, wheat variety, sampling date, ...

-> **frequencies** of each phenotype (%) in sampled populations
• Resistance dynamics

  -> control and treated plots, yield, year, wheat variety, sampling date, ...

  -> frequencies of each phenotype (%) in sampled populations

• Selection pressure

  Proxy: Fungicide use on wheat
Materials – Databases per region

- Resistance dynamics
  
  
  -> control and treated plots, yield, year, wheat variety, sampling date, ...

  -> **frequencies** of each phenotype (%) in sampled populations

- Selection pressure
  
  Proxy: **Fungicide use** on wheat

- Population size
  
  Proxy: Potential **yield losses** induced by *Z. tritici*
• **Resistance dynamics**

  *Resistance monitoring: 2004 - 2017*

  -> control and treated plots, yield, year, wheat variety, sampling date, ...

  -> frequencies of each phenotype (%) in sampled populations

• **Selection pressure**

  Proxy: **Fungicide use** on wheat

• **Population size**

  Proxy: Potential **yield losses** induced by *Z. tritici*

• **Refuges**

  Proxy: Surfaces under **organic wheat**
Methods – Binomial & Zero-One inflated model

\[
Y_{ijkln} = \begin{cases} 
0 & \text{with probability } \pi_0 \\
\sim B(100, p_{ijkln}) & \text{with probability } 1 - \pi_0 - \pi_{100} \\
100 & \text{with probability } \pi_{100}
\end{cases}
\]

- Phenotypes in \textit{emergence} or \textit{counter-selected} → 0%
- Phenotypes in \textit{generalization} → 100%
Methods – A model to quantify resistance evolution

\[ R \text{ resistant phenotype} \quad p_l \text{ proportion of } R \text{ in the population year } l \]
\[ S \text{ sensitive phenotype} \quad q_l = (1 - p_l) \text{ proportion of } S \]

\[ \omega = \omega_R / \omega_S \text{ relative fitness of } R \text{ against } S \]
## Methods – A model to quantify resistance evolution

\[ R \text{ resistant phenotype} \quad p_l \text{ proportion of } R \text{ in the population year } l \]

\[ S \text{ sensitive phenotype} \quad q_l = (1 - p_l) \text{ proportion of } S \]

\[ \omega = \frac{\omega_R}{\omega_S} \text{ relative fitness of } R \text{ against } S \]

<table>
<thead>
<tr>
<th>Time</th>
<th>( R )</th>
<th>( S )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( l - 1 )</td>
<td>( p_{l-1} = \frac{\omega \cdot p_{l-1}}{\omega \cdot p_{l-1} + q_{l-1}} )</td>
<td>( q_{l-1} = \frac{q_{l-1}}{\omega \cdot p_{l-1} + q_{l-1}} )</td>
</tr>
<tr>
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<td>( q_l = \frac{q_{l-1}}{\omega \cdot p_{l-1} + q_{l-1}} )</td>
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</table>

\[ \frac{p_l}{q_l} = \omega \cdot \frac{p_{l-1}}{q_{l-1}} = \omega^2 \cdot \frac{p_{l-2}}{q_{l-2}} = ... = \omega^{l-1} \cdot \frac{p_1}{q_1} \]

\[ \ln\left(\frac{p_l}{1 - p_l}\right) = (l - 1) \ln(\omega) + \ln\left(\frac{p_1}{q_1}\right) \]

\[ e^{\beta} = \omega \]

how many time faster \( R \) grow compared to \( S \)

**\( \mu \)**

initial conditions

\[ \text{logit}(p_l) = \mu + \beta \cdot (l - 1) \]
Methods – A model to quantify resistance evolution

• Descriptive model

\[
\text{logit}(p_{ijn}) = (\mu + \alpha_i) + (\beta + \eta_i) \times (l - 1)
\]

Initial conditions

Constant growth rates \( e^{\beta + \eta_i} = \omega_i \)

With,

\( \mu \): National initial frequency parameter

\( \alpha_i \): Effect of the \( i \)th region on national initial frequency

\( \beta \): National growth rate parameter

\( \eta_i \): Effect of the \( i \)th region on national growth rate

\( l \): Year of the observation, \( l \in [1; L] \), \( L \) is equal to the number of observed years
**Methods – A model to quantify resistance evolution**

- **Descriptive model**

\[
\text{logit}(p_{ijn}) = (\mu + \alpha_i) + (\beta + \eta_i) \times (l - 1) + \delta_j + \varepsilon_{ijblkn}
\]

where \( \varepsilon_{ijln} \sim \mathcal{N}(0, \sigma^2) \)

**Initial conditions**

- **Constant growth rates** \( e^{\beta + \eta_i} = \omega_i \)

**Trial conditions**

- **Noise**

With,

- \( \mu \): National initial frequency parameter
- \( \alpha_i \): Effect of the \( i \)th region on national initial frequency
- \( \beta \): National growth rate parameter
- \( \eta_i \): Effect of the \( i \)th region on national growth rate
- \( l \): Year of the observation, \( l \in [1; L] \), \( L \) is equal to the number of observed years
- \( \delta_j \): Effect of the \( j \)th sampling date
- \( \varepsilon_{ijln} \): Overdispersion
- \( \sigma \): Standard error from the overdispersion
Results – Contrasted rates of evolution

\[ e^\beta + \eta_i \]

(Garnault et al., 2019)
**Results** – Contrast rates of evolution

- Growth rates are different among phenotypes
- **Selection is heterogeneous** through the territory

![Graph showing contrasted rates of evolution](image-url)
Methods – A model to assess resistance evolution determinants

• Explicative model

\[
\logit(p_{ijkln}) = (\mu + \alpha_i) + (\beta + \check{\beta}) \times (l - 1) + \sum_{t=0}^{l-1} \sum_{m=1}^{M} [\nu_m \times F_{itm}] + \rho \times P_{it} + \kappa \times R_{it}) + \delta_j + \gamma_k + \varepsilon_{ijkln}
\]

where \(\gamma_k \sim N(0, \sigma^2_{\text{cultivar}}), \varepsilon_{ijkln} \sim N(0, \sigma^2)\)

and \(F_{0.} = P_{0.} = R_{0.} = 0\)

With,

\(\nu_m\): Effect of the \(m^{th}\) molecule

\(F_{itm}\): Used quantity of the \(m^{th}\) molecule in the \(i^{th}\) region at time \(t\)

\(\rho\): Effect of the population size

\(P_{it}\): Population size in the \(i^{th}\) region at time \(t\)

\(\kappa\): Effect of the refuge surface area

\(R_{it}\): Refuge surface area in the \(i^{th}\) region at time \(t\)

\(\gamma_k\): Effect of the \(k^{th}\) cultivar

\(\sigma_{\text{cultivar}}\): Standard error from the cultivar random effect

Regional **constant** growth

Regional **variable** growth
Methods – A model to assess resistance evolution determinants

• Explicative model

\[
\text{logit}(p_{ijkln}) = (\mu + \alpha_i) \\
+ \left( \beta + \chi \right) \times (l - 1) \\
+ \sum_{t=0}^{l-1} \sum_{m=1}^{M} [\nu_m \times F_{itm}] + \rho \times P_{it} + \kappa \times R_{it} \\
+ \delta_j + \gamma_k \\
+ \varepsilon_{ijkln}
\]

where \( \gamma_k \sim N(0, \sigma^2_{\text{cultivar}}) \), \( \varepsilon_{ijkln} \sim N(0, \sigma^2) \) and \( F_{0.} = P_{0.} = R_{0.} = 0 \)

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- \( \nu_m \): Effect of the \( m \)th molecule
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- \( \gamma_k \): Effect of the \( k \)th cultivar
- \( \sigma_{\text{cultivar}} \): Standard error from the cultivar random effect

Regional constant growth
Regional variable growth

• Associated MoA
• Backward selection
• \( \nu_m > 0 \)
Results – First results: model and factor evaluation

- Model comparison: DIC
  - Explicative model < Descriptive model
    (19378) (20019)

- Factor weighting: $\frac{RSS_{full-\theta}}{RSS_{full}}$
  - Selection pressure > Pop. Size / Refuges
Results – First results: estimation of fungicide effect

- Model comparison
  \[ DIC \]

- Factor weighting
  \[ \frac{RSS_{full - \theta}}{RSS_{full}} \]

- Explicative model < Descriptive model
  (19378) < (20019)

- Selection pressure > Pop. Size / Refuges

- StrR / QoIs (7)

- TriR7-TriR8 ∈ TriMR / DMIs (16)

<table>
<thead>
<tr>
<th>Fungicide ((m))</th>
<th>Estimate ((v_m))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxystrobin</td>
<td>0.26 (.)</td>
</tr>
<tr>
<td>Kresoxim-methyl</td>
<td>0.73 (***</td>
</tr>
<tr>
<td>Pyraclostrobin</td>
<td>0.63 (***</td>
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</tbody>
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<tr>
<th>Fungicide ((m))</th>
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<tr>
<td>Difenconazole</td>
<td>0.32 (**)</td>
</tr>
<tr>
<td>Flusilazole</td>
<td>-0.09</td>
</tr>
<tr>
<td>Hexaconazole</td>
<td>0.12</td>
</tr>
<tr>
<td>Prochloraz</td>
<td>-0.58 (***</td>
</tr>
<tr>
<td>Triadimeno</td>
<td>-0.14</td>
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Results – First results: estimation of fungicide effect

- Model comparison
  - \( DIC \)

- Factor weighting
  - \( \frac{RSS_{full-\theta}}{RSS_{full}} \)

Explicative model < Descriptive model
(19378) (20019)

Selection pressure > Pop. Size / Refuges

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➢ TriR7-TriR8 ∈ TriMR / DMIs (16)

(Walker, 2011)
Conclusion – Take home messages

• Large-scale monitoring allows quantitative description of resistance dynamics.

• We highlighted heterogeneity of resistance evolution among phenotypes.

• Heterogeneity can be explained in majority by regional fungicide uses.

• Estimations are consistent with previous description of phenotypes.
Thank you for your attention

Special thanks to:

AMAR research team

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Florence Carpentier

Olivier David

Christian Lannou

Gilles Couleaud

Clarisse Payet

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c SPE Department, INRA, France

d ARVALIS Institut du Végétal

e BAYER Crop Science


Very **low use** of benzimidazoles

Average frequency remains around **90%**

**Zero-cost resistance?**
Quinone outside Inhibitors

Average frequency remains > 95%

Very fast evolution after emergence

Low-cost resistance + strong selection pressure?
No more sensitive phenotype since the mid-1990s

Sequential emergence of TriLR, TriMR and TriHR and MDR
Qualitative resistance to DMIs

- Poly-allelism
- Multigenic

Numerous phenotypes

1 phenotype = 1 resistance spectrum

<table>
<thead>
<tr>
<th>IDM</th>
<th>TriR1/R3</th>
<th>TriR2/R4</th>
<th>TriR5</th>
<th>TriR6</th>
<th>TriR7/R8</th>
<th>TriR5+</th>
<th>TriR8+</th>
<th>TriR9</th>
<th>TriR10/R11</th>
<th>MDR</th>
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<tr>
<td>Pyrifenox</td>
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<tr>
<td>Classe phénotypique</td>
<td>TriLR</td>
<td>TriMR</td>
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</table>

*(Walker, 2011)*
Appendix

Spatial model
GLMM with Matèrn covariance
Kriging + Spatial partitioning
Maps of resistance status

ANOVA-like model
Region x Year
Highlighting deviations from the average
Quantify observations on maps

Dynamic
- StrR: 120 à 150 km/year

Static
- TriR6: N/N-E (NPC, IDF)
- TriR7-TriR8: S/S-W (MPY, AQU, PCH)

(Garnault et al., 2019)
Yield: 70 q/ha
Area under wheat: 5 000ha
Production: 35 000t
Internal market 15 000t
Stocks: 2 500t
Foreign market: 20 000t
Market price: 175€/t
Total: 3,5 Md€

Yield: 50 q/ha
Area under wheat: 5 000ha
Production: 25 000t
Internal market 15 000t
Stocks: 2 500t
Foreign market: 10 000t
Market price: 175€/t
Total: 1,7 Md€

≈ -20 q/ha
≈ -2 Md€