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

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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)

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Purpose

Better **management of resistance** in agriculture

Need to increase our capacity to **predict the dynamics** of resistance

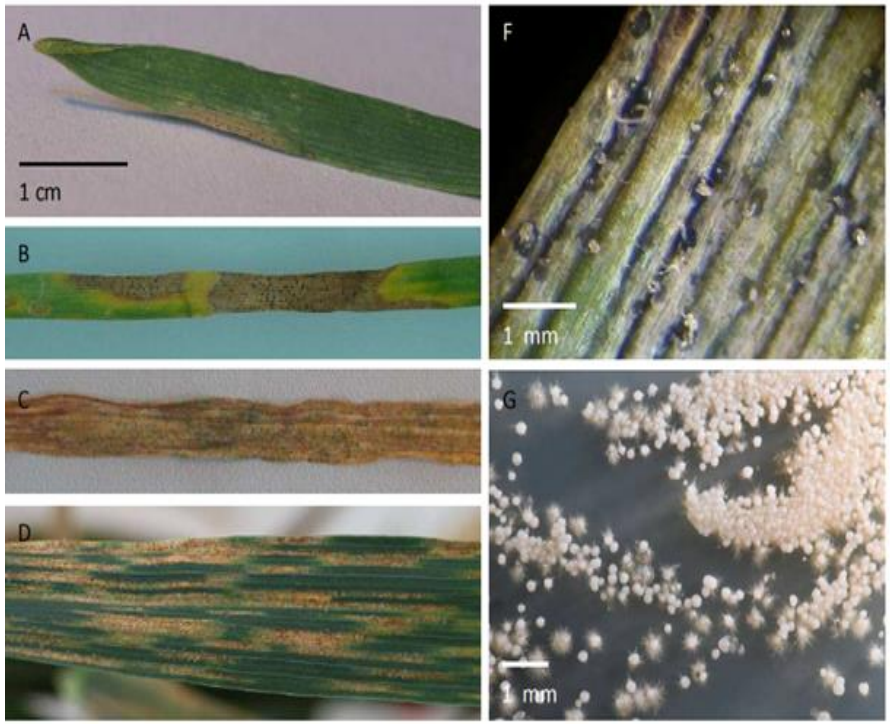
Retrospectively study the **spatio-temporal evolution of resistance:**

- **Is the evolution of resistance homogeneous in France ?**
- **Is the regional fungicide use a major driver of resistance selection ?**

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

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

(Fones & Gurr, 2015)



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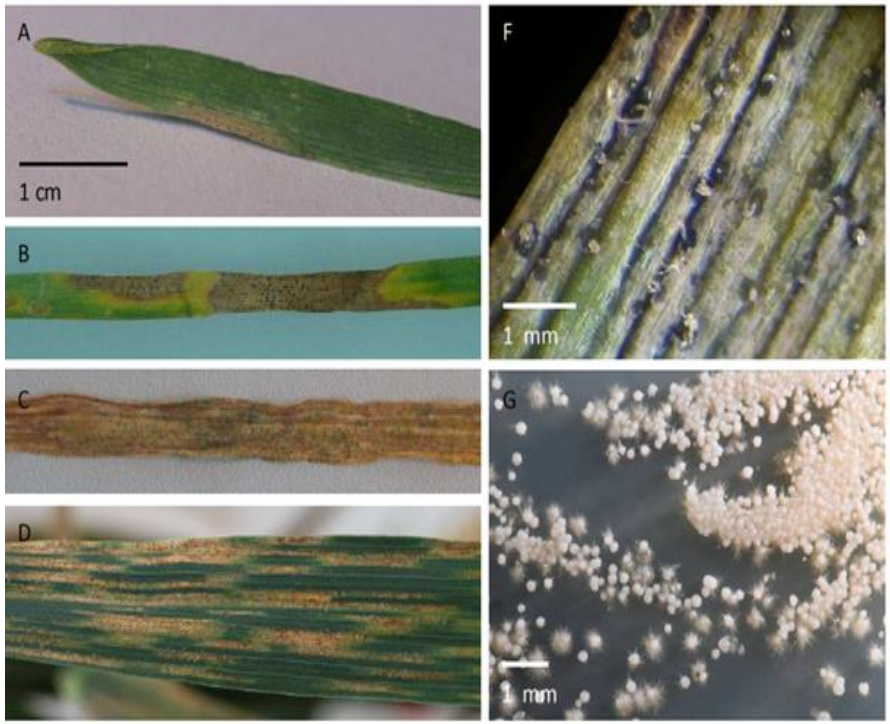

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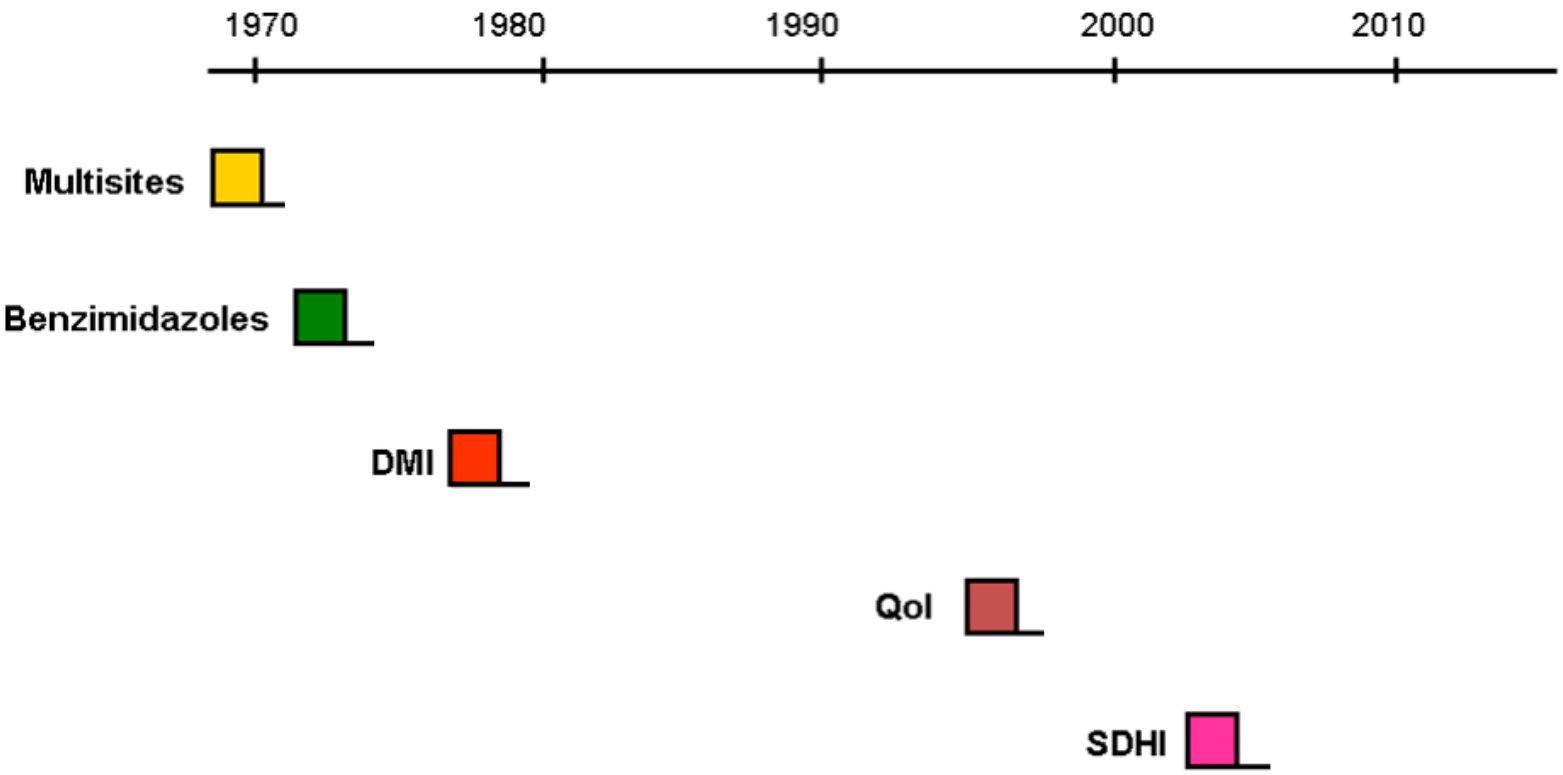

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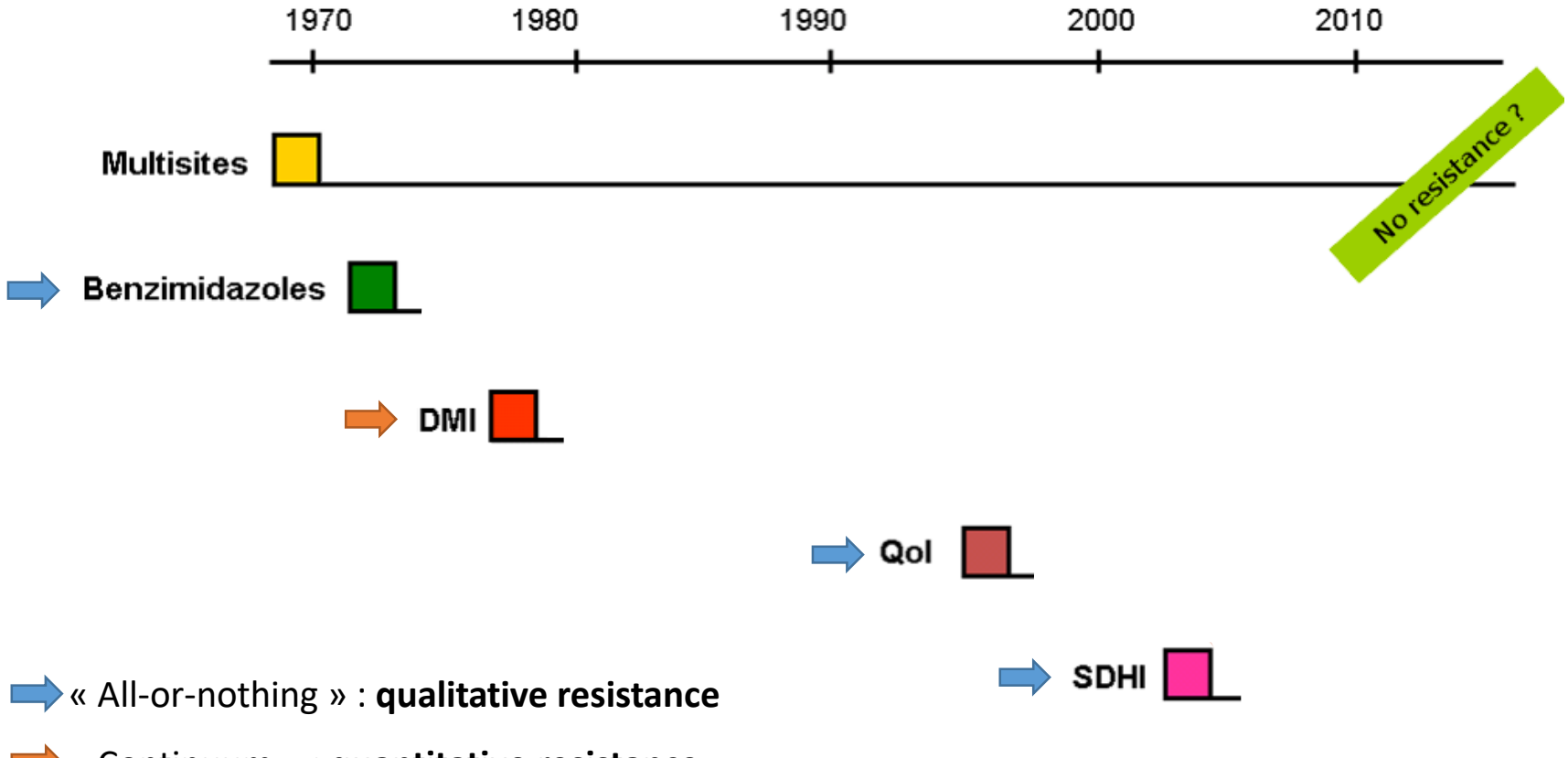

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« Continuum » : **quantitative resistance**

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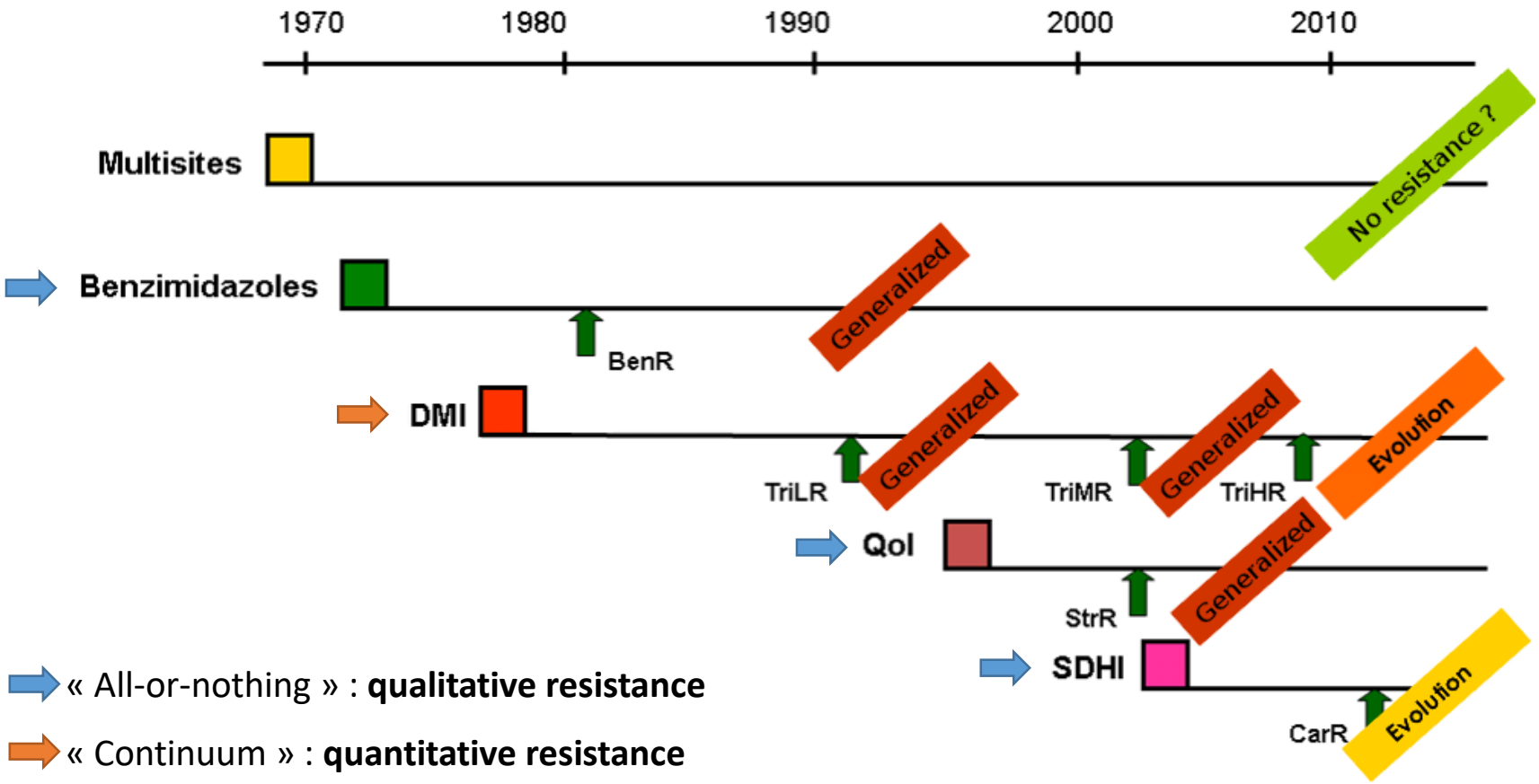
- Some resistances are **well established in populations**



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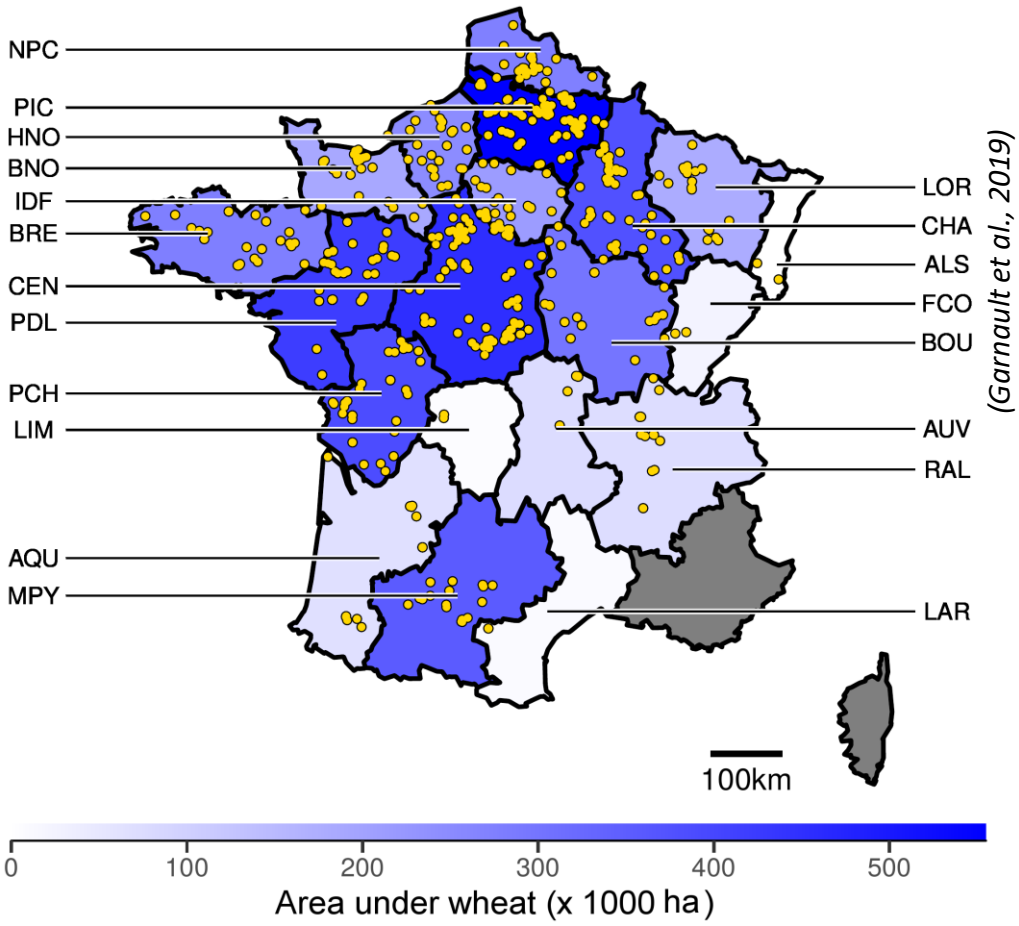
Materials – Databases per region

- **Resistance dynamics**

Resistance monitoring: 2004 - 2017

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

1013 trials



(Garnault et al., 2019)

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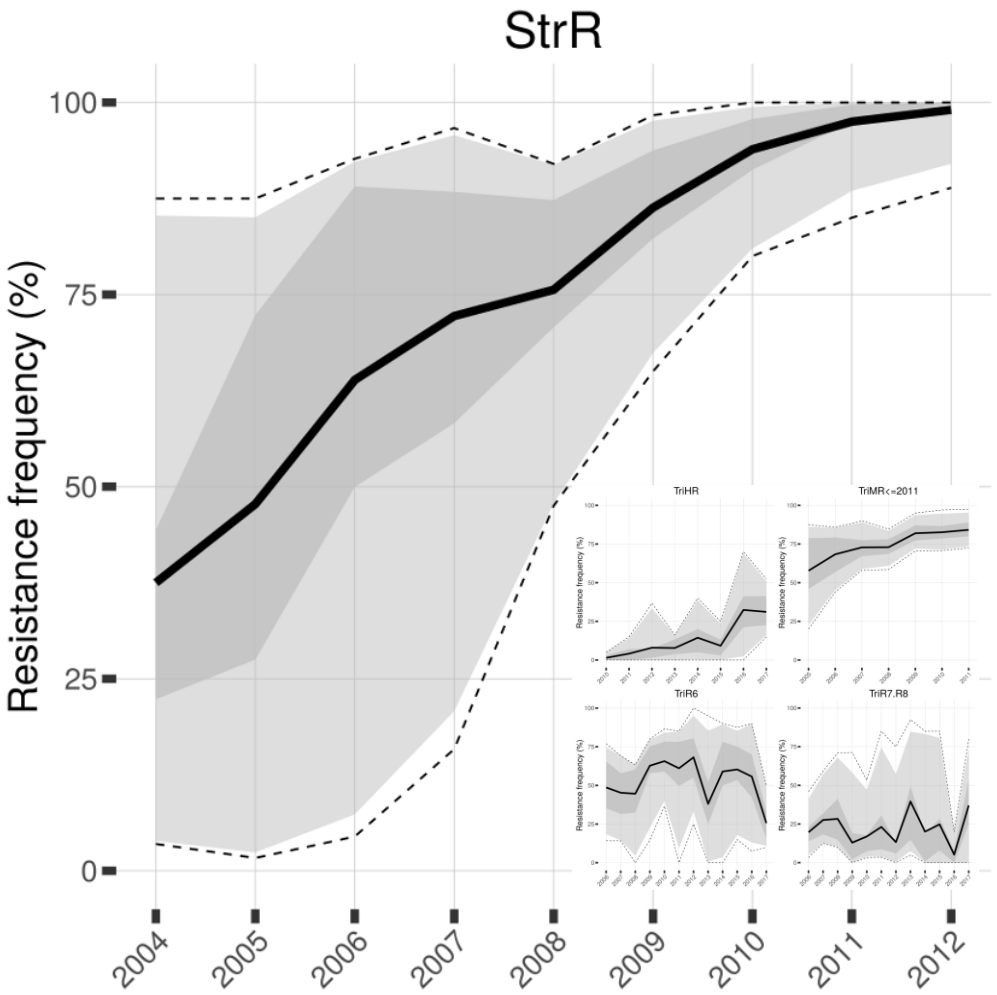

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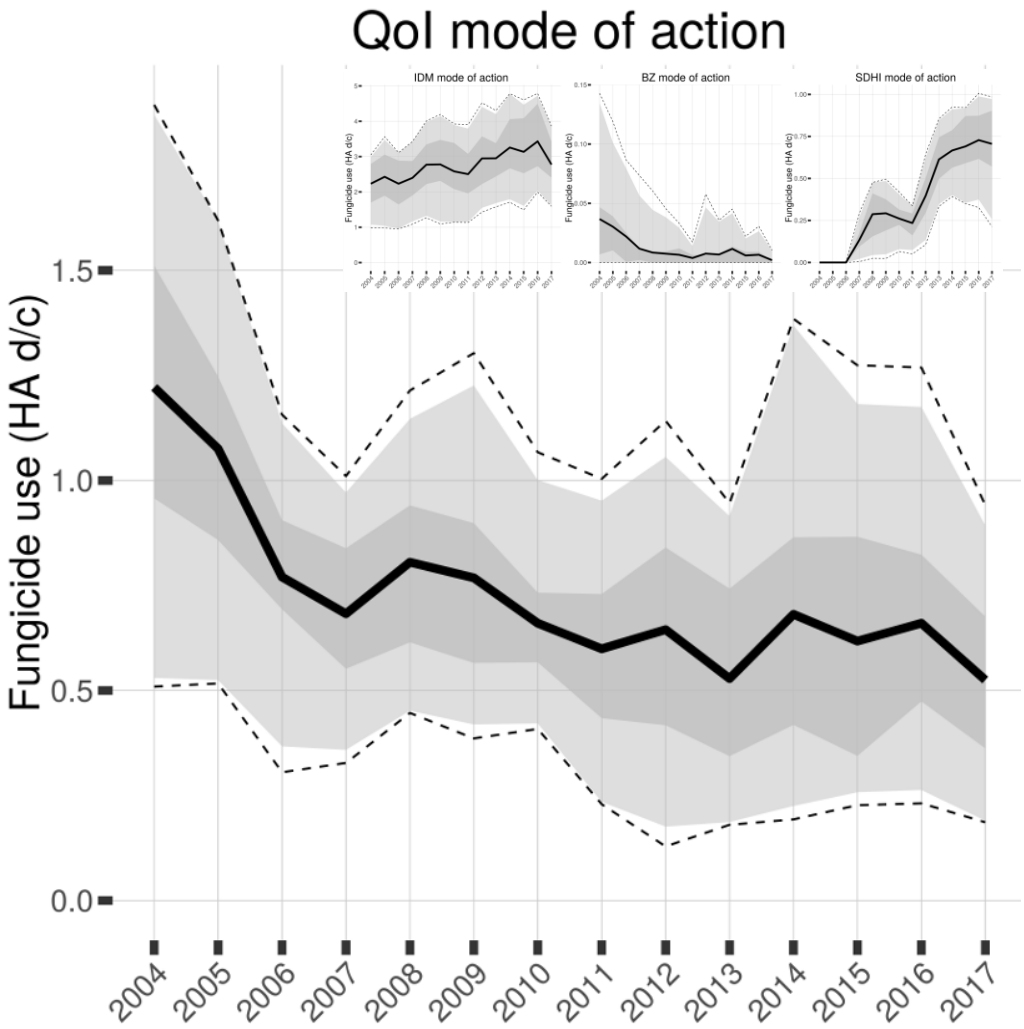
  Proxy: **Fungicide use** on wheat



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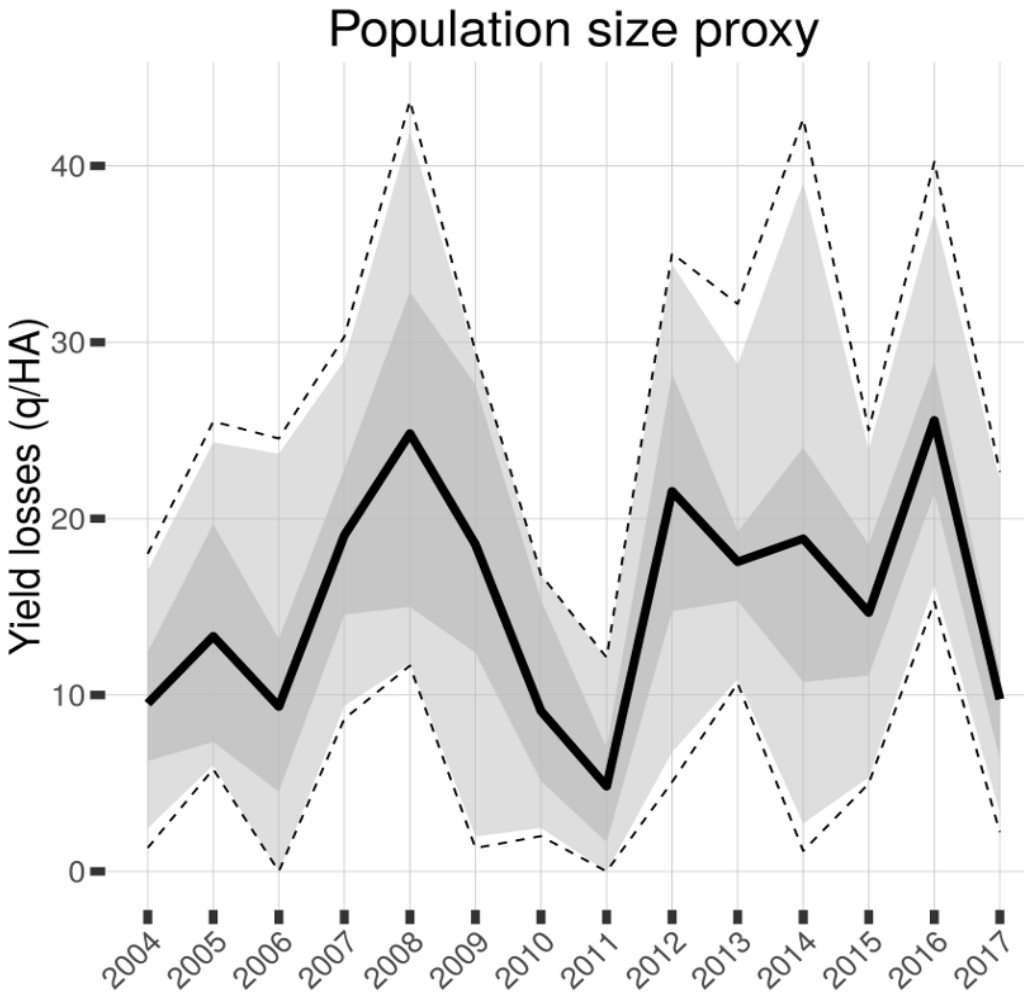
  Proxy: Potential **yield losses** induced by *Z. tritici*



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- **Resistance dynamics**

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- **Selection pressure**

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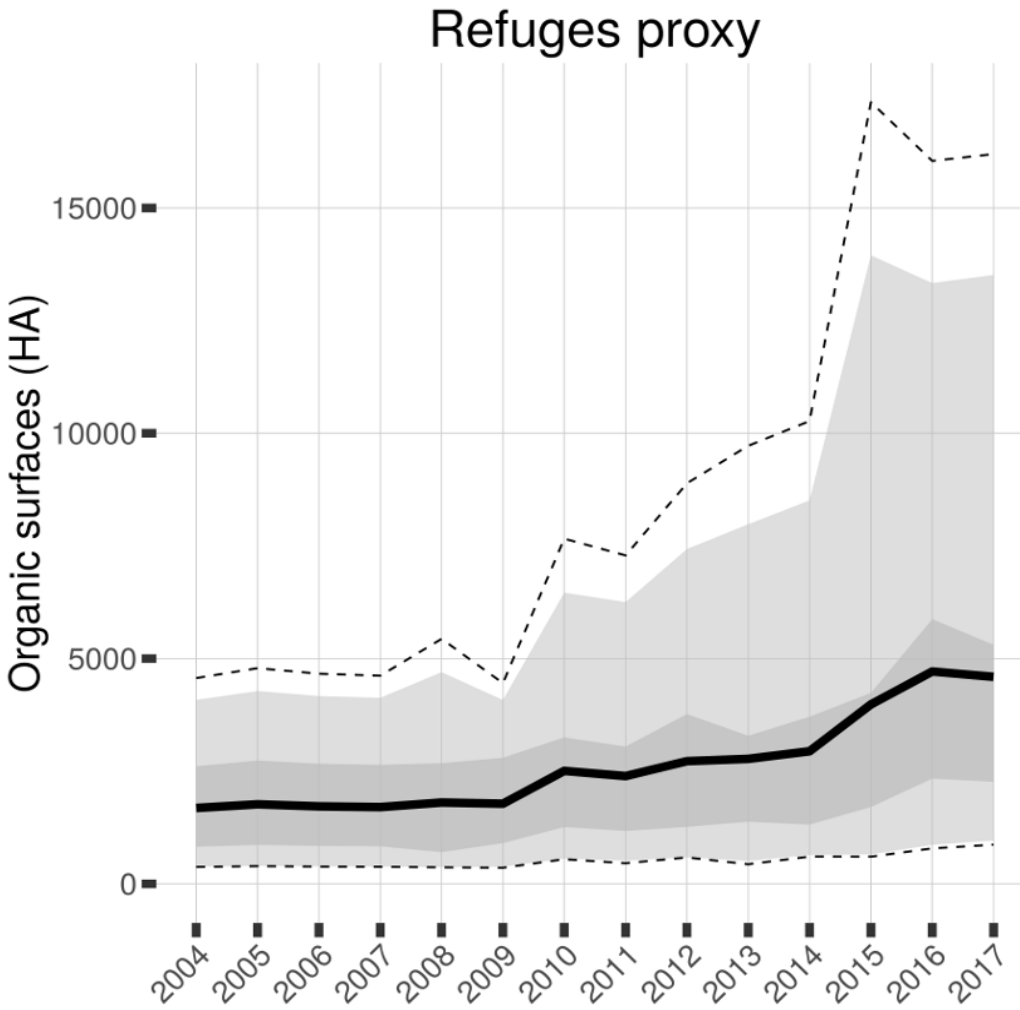
- **Population size**

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

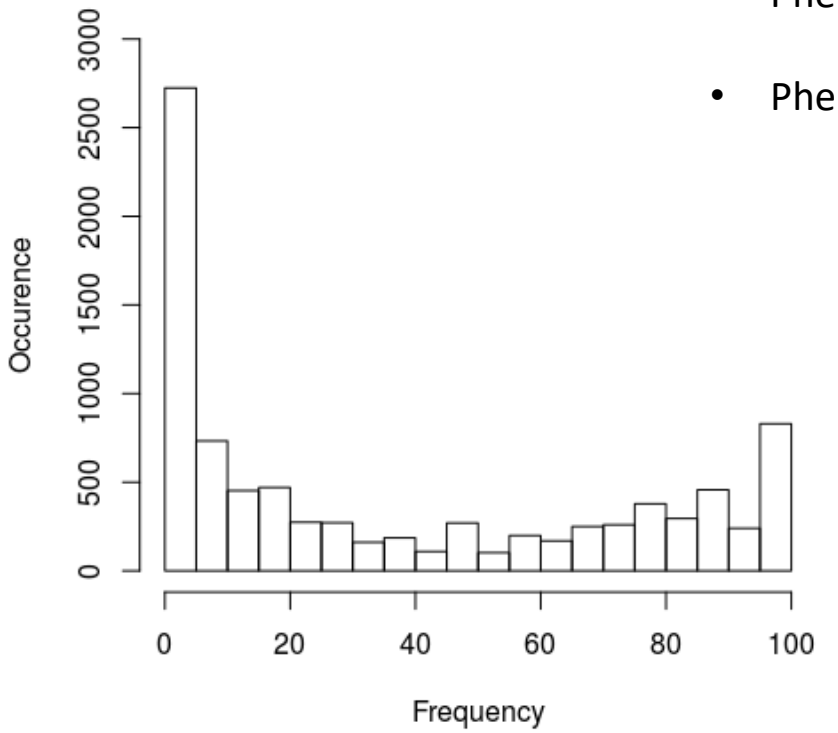
- **Refuges**

  Proxy: Surfaces under **organic wheat**



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

- Phenotypes in **emergence** or **counter-selected** ➡ 0%
- Phenotypes in **generalization** ➡ 100%



(Ntzoufras, 2011; Ospina and Ferrari, 2012)

Methods – A model to quantify resistance evolution

R resistant phenotype — p_l proportion of R in the population year l

S sensitive phenotype — $q_l = (1 - p_l)$ proportion of S

$\omega = \omega_R/\omega_S$ relative fitness of R against S

(Hartl and Clark, 1997)

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	$Time$	R	S
Proportion before selection	$l-1$	p_{l-1}	q_{l-1}
Proportion after selection	l	$p_l = \frac{\omega * p_{l-1}}{\omega * p_{l-1} + q_{l-1}}$	$q_l = \frac{q_{l-1}}{\omega * p_{l-1} + q_{l-1}}$

(Hartl and Clark, 1997)

$$\frac{p_l}{q_l} = \omega * \frac{p_{l-1}}{q_{l-1}} = \omega^2 * \frac{p_{l-2}}{q_{l-2}} = \ldots = \omega^{l-1} * \frac{p_1}{q_1}$$

$$ln(\frac{p_l}{1-p_l}) = (l-1) * ln(\omega) + ln(\frac{p_1}{q_1})$$

$$logit(p_l) = \mu + \beta * (l-1)$$

$\boldsymbol{\mu}$
initial conditions

$\boldsymbol{e^{\beta} = \omega}$
how many time faster *R* grow compared to *S*

- **Descriptive model**

$$logit(p_{ijln}) = (\mu + \alpha_i) + (\beta + \eta_i) * (l - 1)$$

Initial conditions

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

With,
μ : National initial frequency parameter
α_i: Effect of the i^{th} region on national initial frequency

β: National growth rate parameter
η_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

(Garnault et al., 2019)

- **Descriptive model**

$$logit(p_{ijln}) = (\mu + \alpha_i) \quad \text{Initial conditions}$$

$$+ (\beta + \eta_i) * (l - 1) \quad \text{Constant growth rates} \quad e^{\beta+\eta_i} = \omega_i$$

$$+ \boxed{\delta_j} \quad \text{Trial conditions}$$

$$+ \boxed{\varepsilon_{ijkln}} \quad \text{Noise}$$

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

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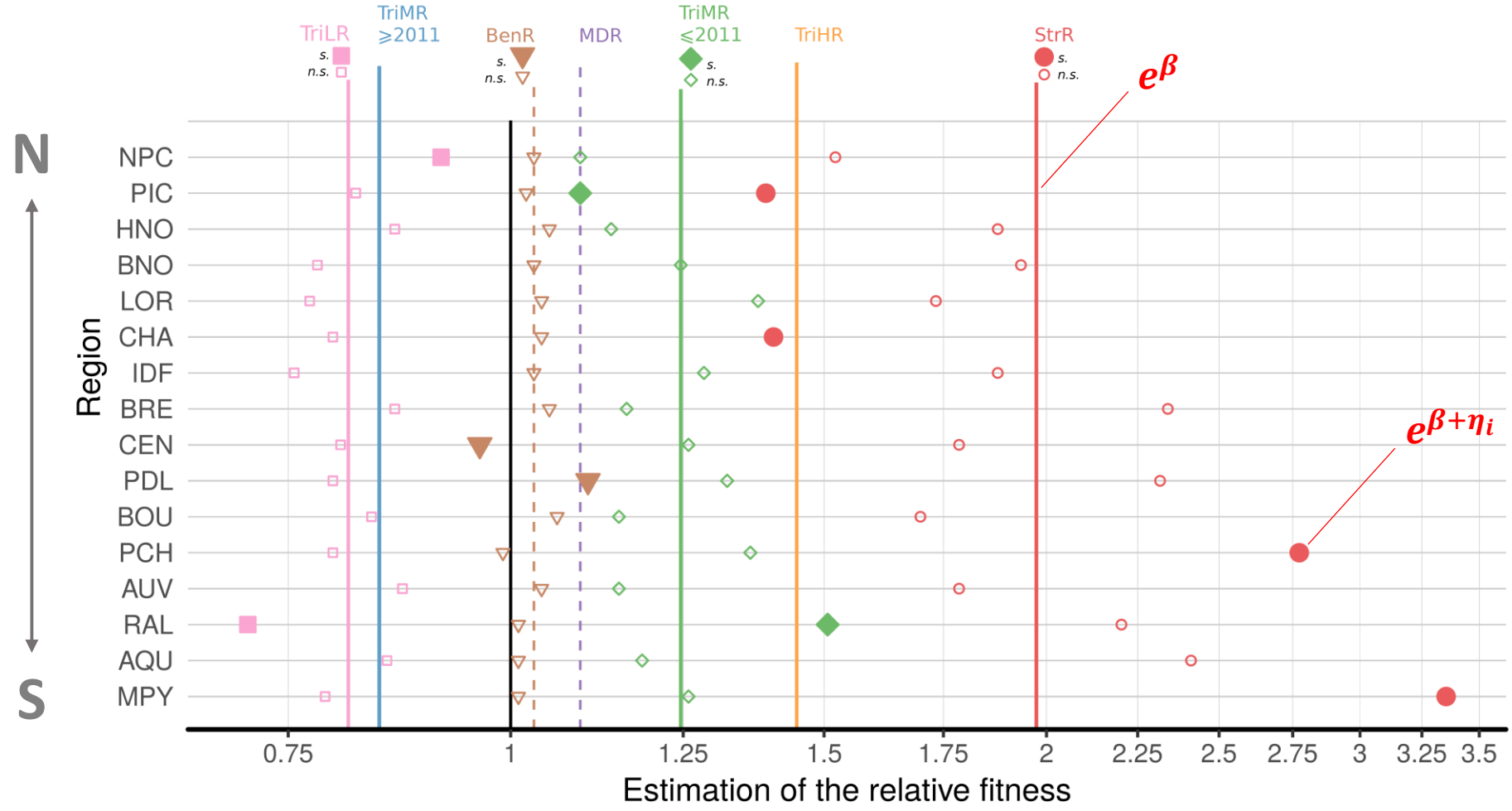
δ_j: Effect of the j^{th} sampling date

ε_{ijln}: Overdispersion
σ: Standard error from the overdispersion

(Garnault et al., 2019)

Results – Contrasted rates of evolution



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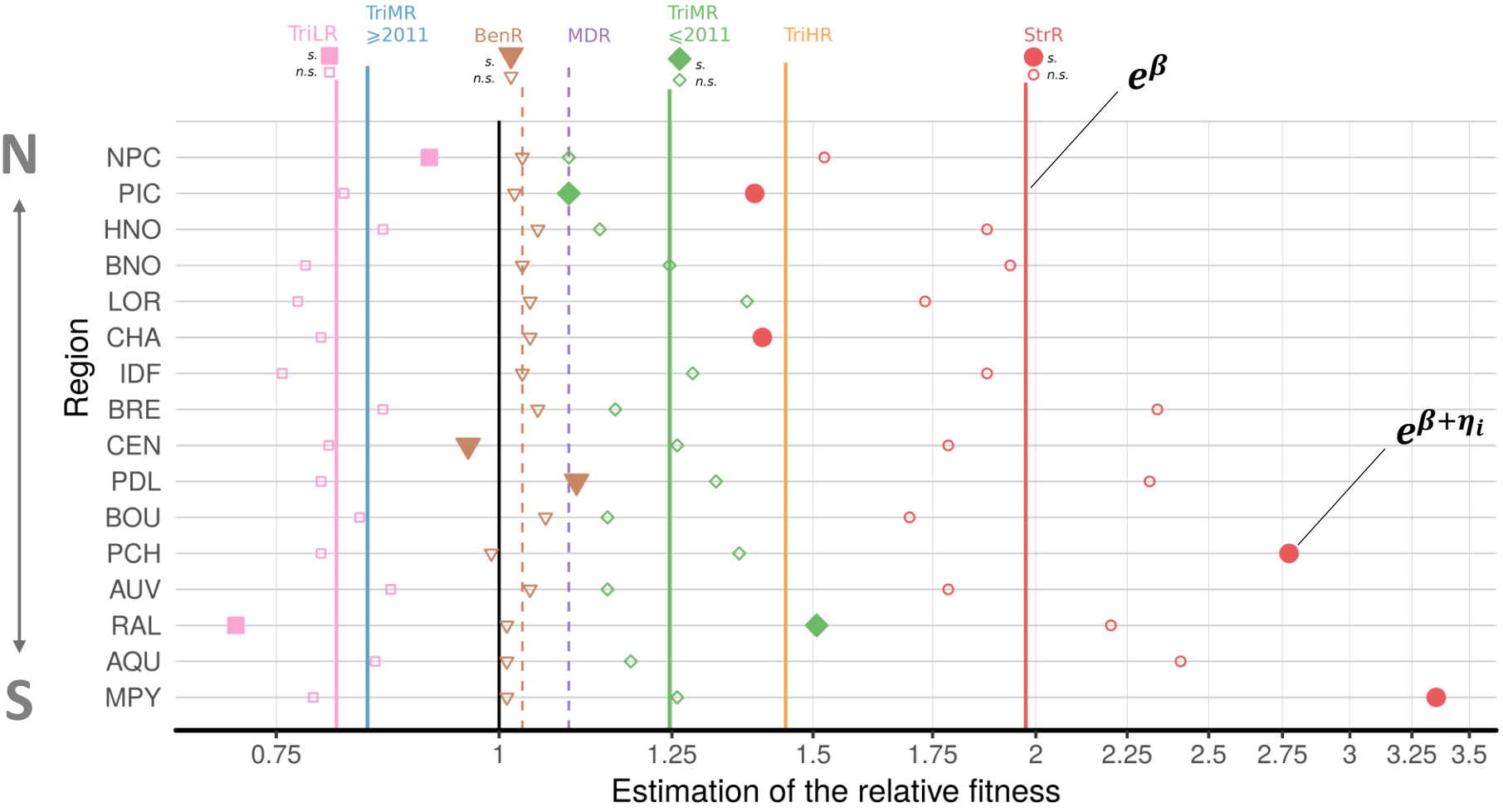

(Garnault et al., 2019)

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

- **Explicative model**

$$
\begin{aligned}
logit(p_{ijkln}) = \; & (\mu + \alpha_i) \\
& + (\beta + \cancel{\gamma_i}) * (l-1) \\
& + \boxed{\sum_{t=0}^{l-1} \Big(\sum_{m=1}^{M} [\nu_m * F_{itm}] + \rho * P_{it} + \kappa * R_{it} \Big)} \\
& + \delta_j + \gamma_k \\
& + \varepsilon_{ijkln}
\end{aligned}
$$

where $\gamma_k \underset{iid}{\sim} \mathcal{N}(0,\ \sigma^2_{cultivar})$, $\varepsilon_{ijkln} \underset{iid}{\sim} \mathcal{N}(0,\ \sigma^2)$
and $F_{\cdot 0 \cdot} = P_{\cdot 0} = R_{\cdot 0} = 0$

Regional **constant** growth

⇩

Regional **variable** growth

With,

ν_m: Effect of the m^{th} molecule
F_{itm}: Used quantity of the m^{th} molecule in the i^{th} region at time t
ρ: Effect of the population size
P_{it}: Population size in the i^{th} region at time t
κ: Effect of the refuge surface area
R_{it}: Refuge surface area in the i^{th} region at time t

γ_k: Effect of the k^{th} cultivar
$\sigma_{cultivar}$: Standard error from the cultivar random effect

- **Explicative model**

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⇒
- **Associated MoA**
- **Backward selection**
- $\boldsymbol{\nu_m > 0}$

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 **Explicative model (19378)** < Descriptive model (20019)
- **Factor weighting** $\frac{RSS_{full-\theta}}{RSS_{full}}$ **Selection pressure** > Pop. Size / Refuges

➢ **StrR / QoIs (7)**

Fungicide (m)	**Estimate (ν_m)**
Fluoxystrobin	0.26 (.)
Kresoxim-methyl	**0.73 (***)**
Pyraclostrobin	**0.63 (***)**

➢ TriR7-TriR8 ∈ **TriMR / DMIs (16)**

Fungicide (m)	**Estimate (ν_m)**
Difenconazole	**0.32 (**)**
Flusilazole	-0.09
Hexaconazole	0.12
Prochloraz	**-0.58 (***)**
Triadimenol	-0.14

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(Walker, 2011)

	TriLR	TriMR		TriHR
		TriR6	TriR7-TriR8	
Pyrifenox				
Prochloraz				
Epoxiconazole				
Propiconazole				
Fluquinconazole				
Tebuconazole				
Metconazole				
Difenconazole				
Prothioconazole				

Resistance factor			
Sensitive	Low	Medium	High

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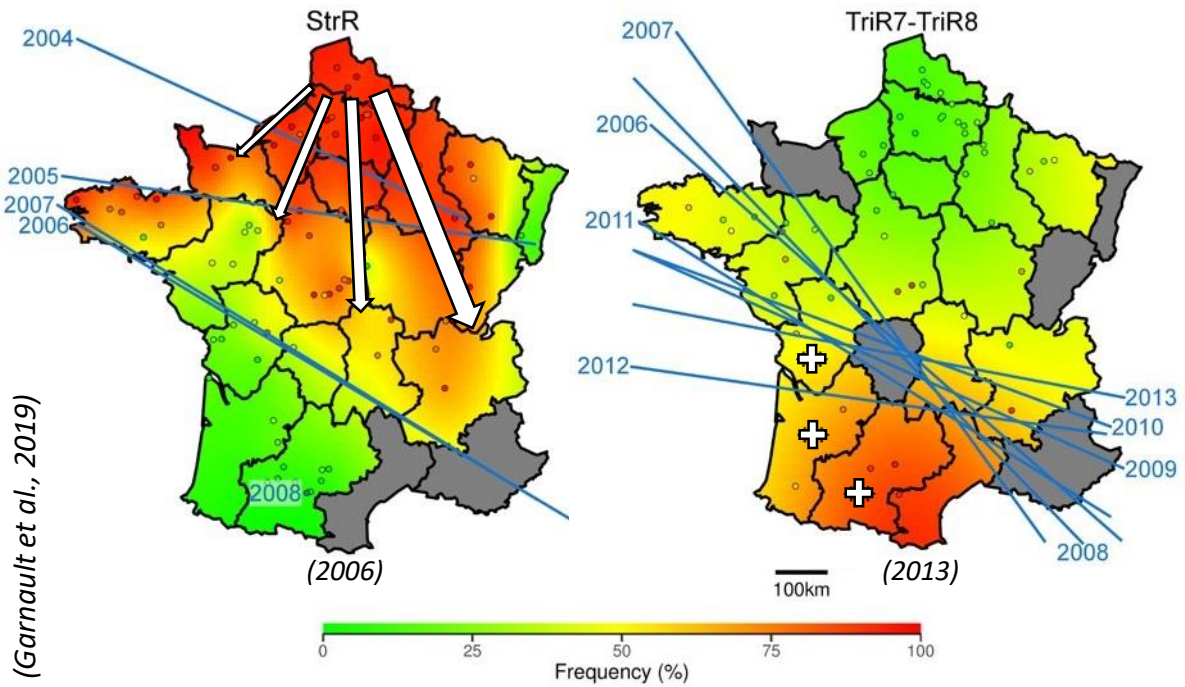
Conclusion – Take home messages

- 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



(Garnault et al., 2019)

Special thanks to:

AMAR research team[a]

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[c] SPE Department, INRA, France
[d] ARVALIS Institut du Végétal
[e] BAYER Crop Science

References

Grimmer, M. K., van den Bosch, F., Powers, S. J., & Paveley, N. D. (2015). Fungicide resistance risk assessment based on traits associated with the rate of pathogen evolution. Pest management science, 71(2), 207-215.

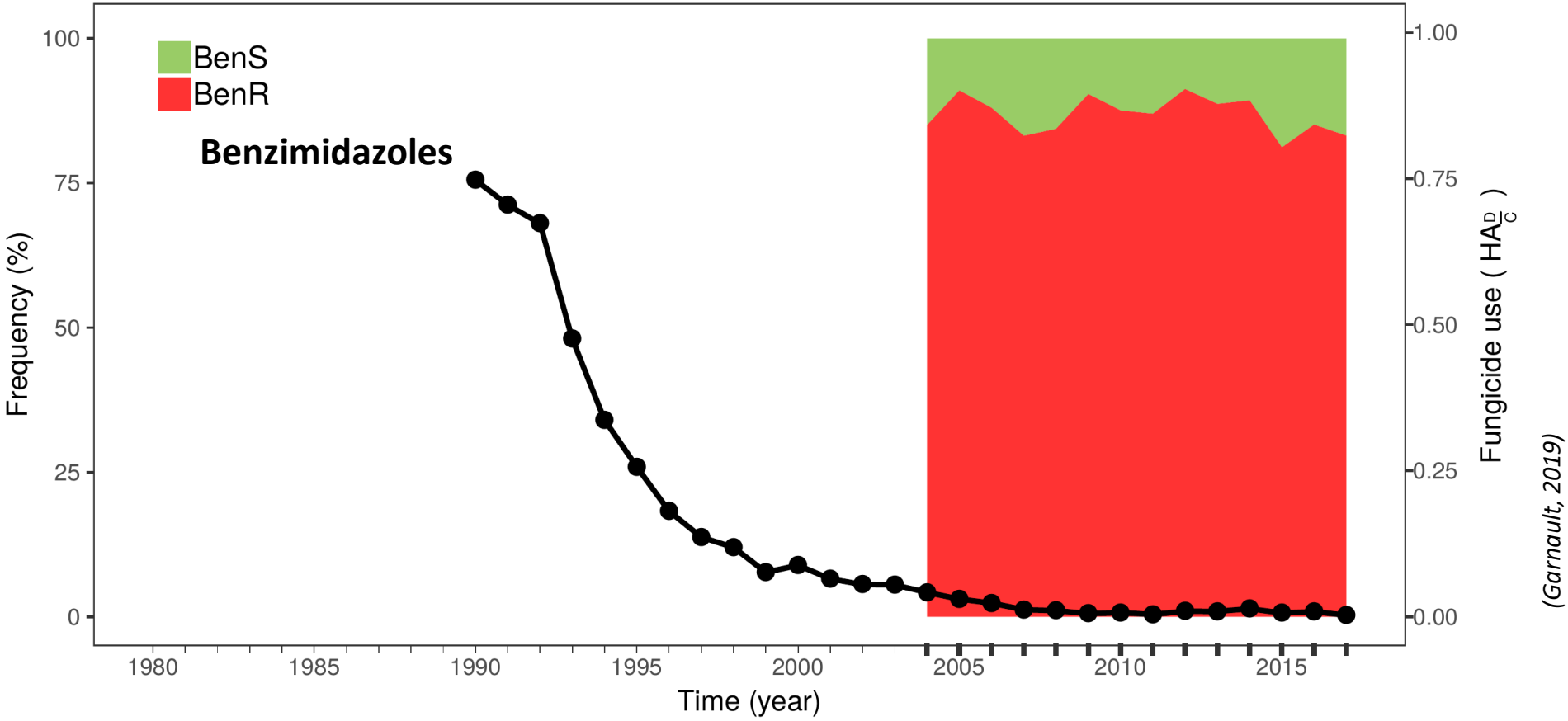
Morais, D. (2015). Components of the early stages of Septoria tritici blotch epidemics (*Zymoseptoria tritici*): quantity, efficiency and origin of primary inoculum.

Huf, A., Rehfus, A., Lorenz, K. H., Bryson, R., Voegele, R. T., Stammler, G. Proposal for a new nomenclature for CYP 51 haplotypes in *Zymoseptoria tritici* and analysis of their distribution in Europe. Plant Pathology. 10.1111/ppa.12891

Torriani S. F., Brunner P. C., McDonald B. A., Sierotzki H. (2009). QoI resistance emerged independently at least 4 times in European populations of Mycosphaerella graminicola, Pest Manag Sci 65:155–162.

Leroux, P. and Walker, A. S. (2011). Multiple mechanisms account for resistance to sterol 14α-demethylation inhibitors in field isolates of Mycosphaerella graminicola. Pest Management Science, 67(1), 44-59.

Appendix

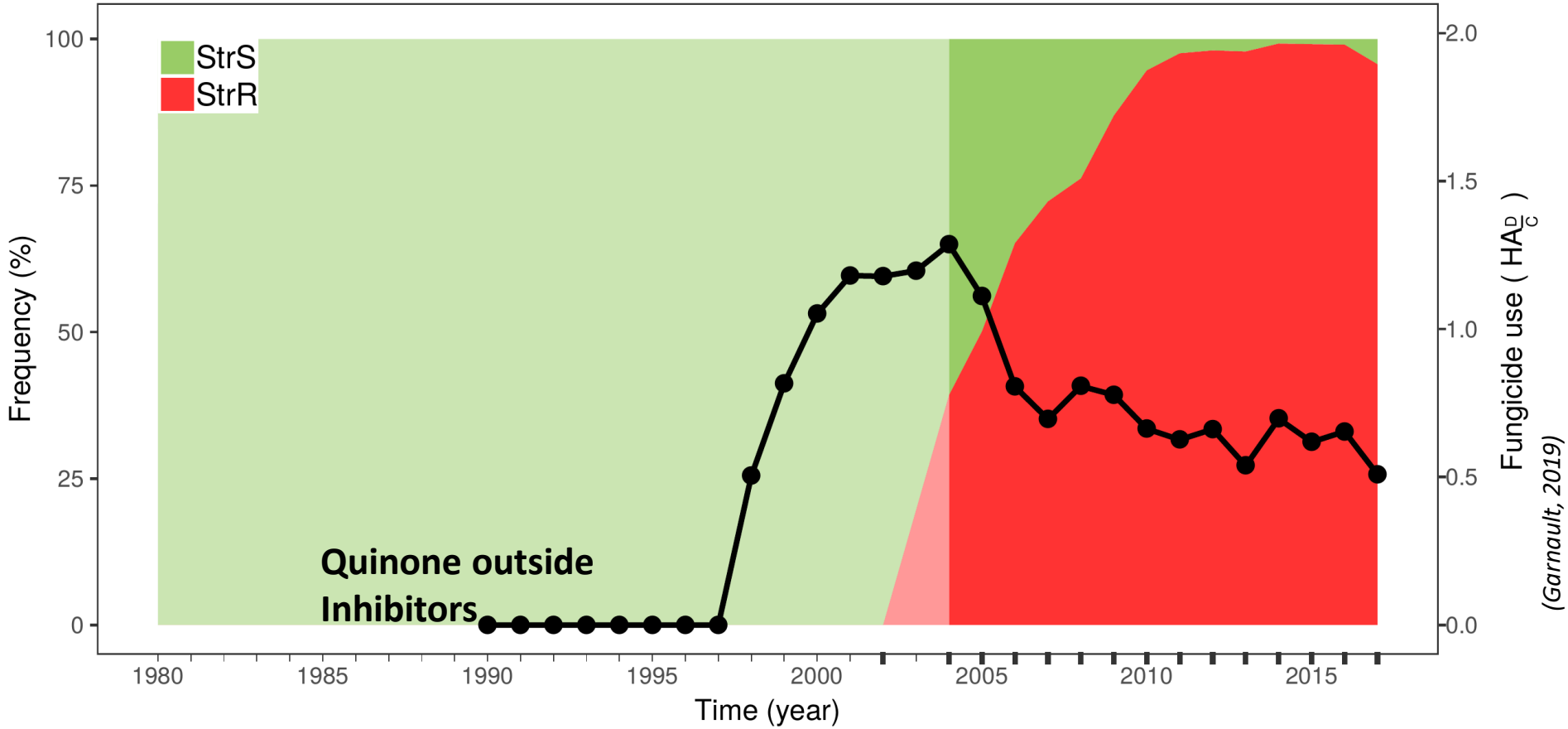


Very **low use** of benzimidazoles

Average frenquency remains around **90%**

Zero-cost resistance ?

Appendix

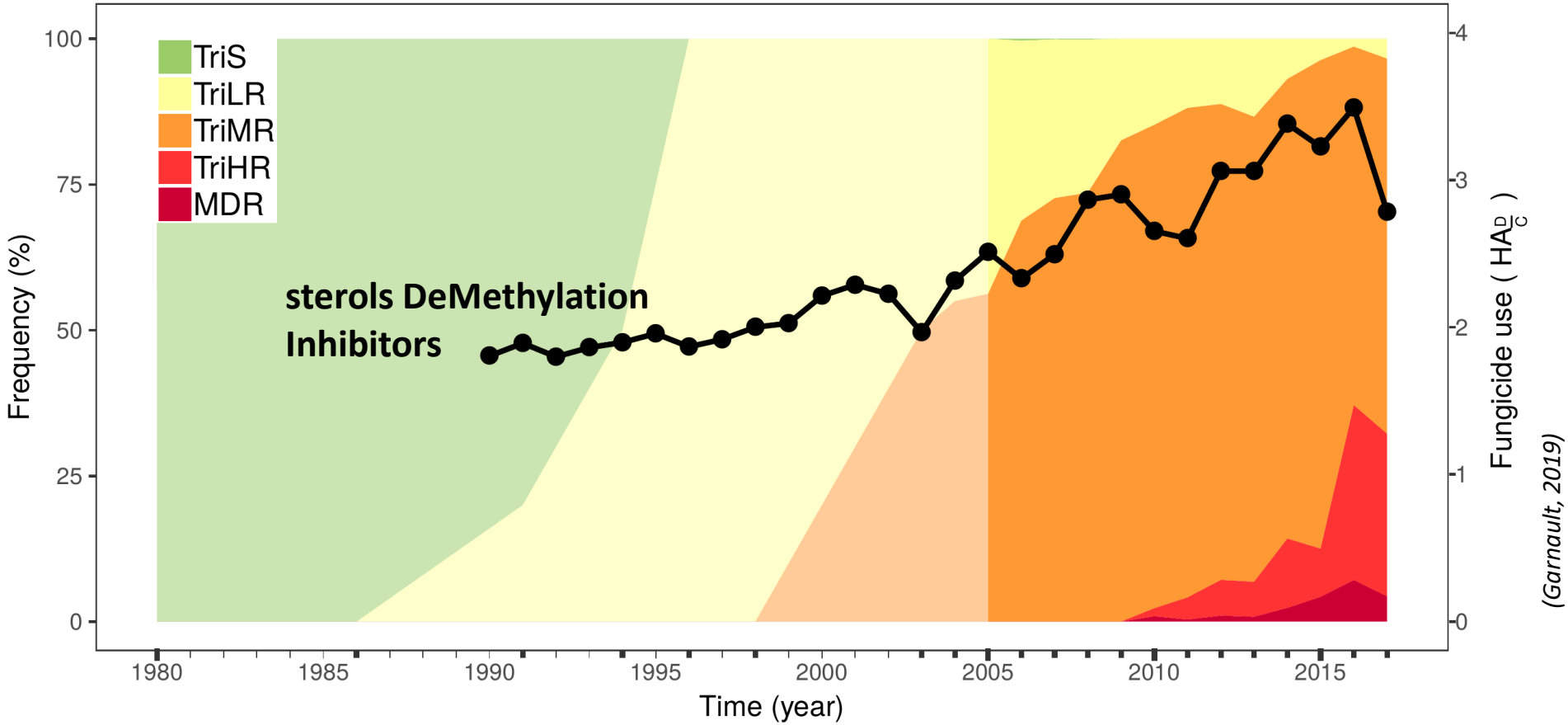


Average frenquency remains **> 95%**

Very fast evolution after emergence

Low-cost resistance + strong selection pressure ?

Appendix



No more sensivite 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**

IDM	TriR1 /R3	TriR2 /R4	TriR5	TriR6	TriR7 /R8	TriR5 +	TriR8 +	TriR9	TriR10 /R11		MDR
Pyrifenox										En cours de caractérisation	
Prochloraze											
Epoxiconazole											
Propiconazole											
Fluquinconazole											
Tébuconazole											
Metconazole											
Difénoconazole											
Prothioconazole											
Classe phénotypique	TriLR			TriMR		TriHR					

Niveau de résistance

0.5-2.5	2.5-25	25-100	>100
Sensible	Faible	Moyen	Fort

(Walker, 2011)

Spatial model

GLMM with **Matèrn** covariance

↓

Kriging + Spatial partitioning

↓

Maps of resistance status

⟷

ANOVA-like model

Region x Year

↓

Highlighting **deviations from the average**

↓

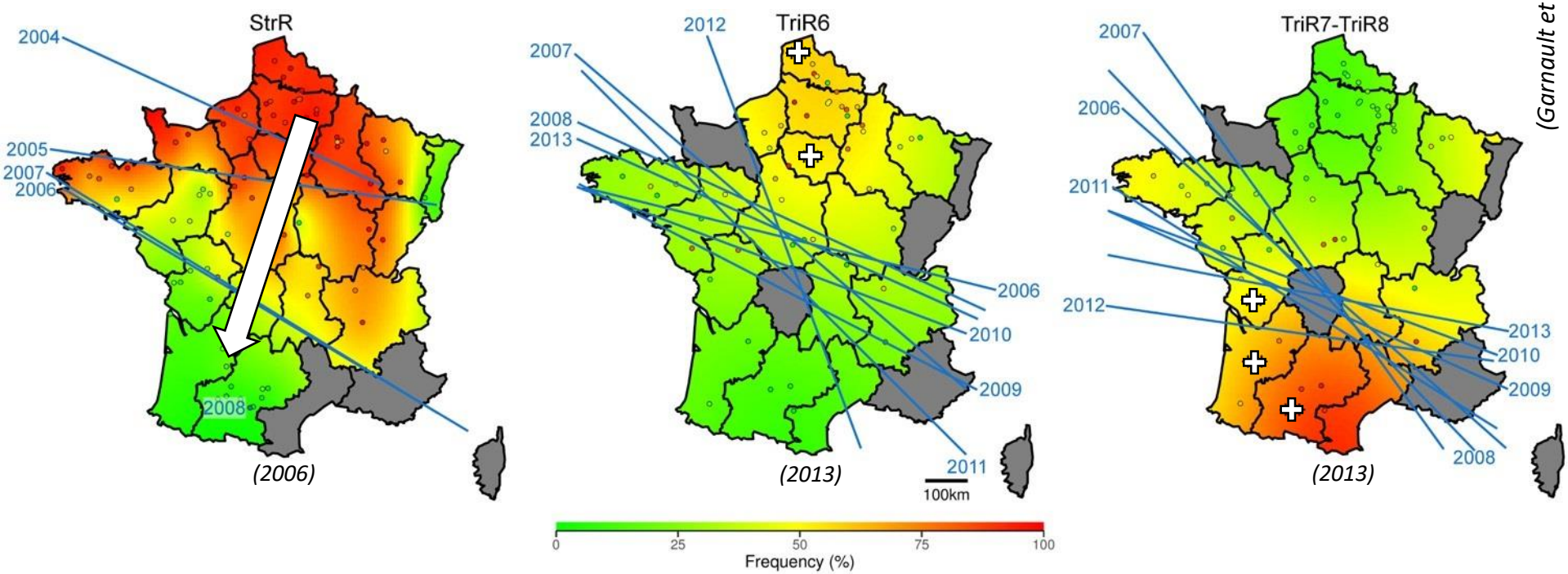
Quantify observations on maps

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)

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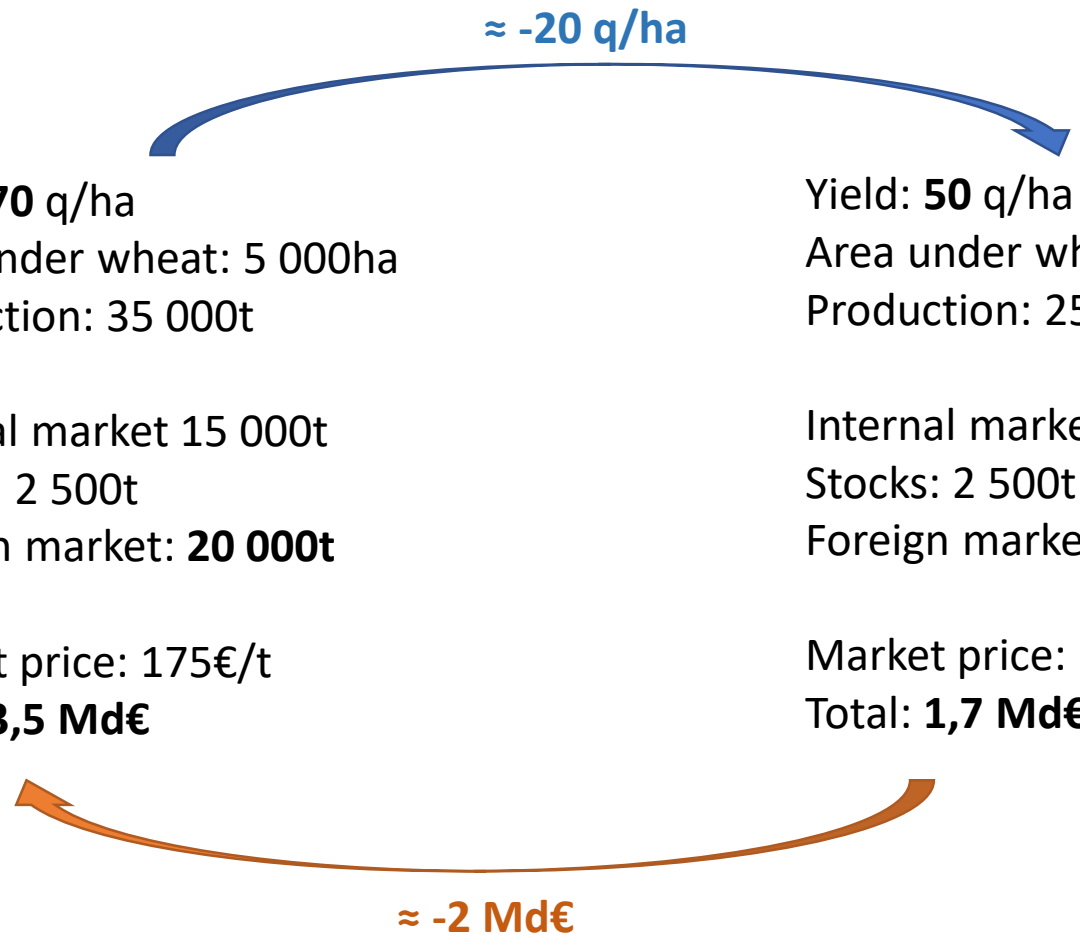

(FranceAgriMer, 2016; Arvalis Institut du végétal, 2017)