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1 **Large-scale study reveals regional fungicide applications as a major determinant of**
2 **resistance evolution in the wheat pathogen *Zymoseptoria tritici* in France.**

3

4 **Running head:**

5 Resistance selection driven by regional fungicide use in French *Zymoseptoria tritici*
6 populations.

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22 **Summary:**

- 23 • Research rationale: In modern cropping systems, the quasi-systematic use of plant
24 protection products selects for resistance in pest populations. The emergence and
25 evolution of this adaptive trait threaten treatment efficacy. We identified determinants
26 of fungicide resistance evolution and quantified their effects at a large spatiotemporal
27 scale.
- 28 • Methods: We focused on *Zymoseptoria tritici*, which causes leaf blotch in wheat.

29 Phenotypes of qualitative or quantitative resistance to various fungicides were
30 monitored annually, from 2004 to 2017, at about 70 sites throughout regions of France
31 (territorial units of 25 000km² in average). We modelled changes in resistance
32 frequency with regional anti-*Septoria* fungicide use, yield losses due to the disease and
33 the regional area under organic wheat.

- 34 • Key results: The major driver of resistance dynamics was fungicide use at the regional
35 scale. We estimated its effect on the increase in resistance and apparent relative fitness
36 of each resistance phenotype. The predictions of the model replicated the
37 spatiotemporal patterns of resistance observed in field populations (R^2 from 0.56 to
38 0.82).
- 39 • Main conclusion: The evolution of fungicide resistance is determined at the regional
40 scale. There is therefore a need for the collective management of resistance, at local
41 but also non-intuitively at larger scales, which could be guided by the results of studies
42 like this.

43

44 **Keywords:**

45 *Mycosphaerella graminicola*, *Zymoseptoria tritici*, Septoria leaf blotch,

46 Resistance determinants, Large-scale evolution, Regional fungicide use, Resistance
47 dynamics, Mathematical modelling

48

49 **1 INTRODUCTION**

50 The efficacy of pesticides and drugs has been compromised by the rapid and widespread
51 evolution of resistance, increasing the use of pesticides and drugs to maintain control levels
52 (Georghiou & Mellon, 1983; Russell, 2005; Gould *et al.*, 2018). The management of resistance
53 evolution is essential for human health, biodiversity and food security, given the rapid
54 emergence and spread of resistance and the lack of new modes of action (MoA) (Palumbi,
55 2001; Grimmer *et al.*, 2014). Many studies have investigated the effects of various factors on
56 the evolution of resistance: fitness cost (Andersson, 2003), mutation rate (Martinez & Baquero,

57 2000; Gressel, 2011), population size (Sisterson *et al.*, 2004), strength of selection pressure
58 and its mitigation in anti-resistance strategies (Oz *et al.*, 2014; van den Bosch *et al.*, 2014). A
59 number of studies have advocated further studies on the relative impact of these factors on a
60 given pest and of the interactions between these factors (Berendonk *et al.*, 2015; Hughes &
61 Andersson, 2015), with a view to promoting large-scale strategies (Okeke *et al.*, 2005;
62 Menalled *et al.*, 2016).

63

64 Several studies have shown how agricultural selection pressures affect the large-scale
65 structure of pest populations at national scale. For instance, the national distribution of
66 resistance varieties shapes the adaptation of pathogen populations to cultivars (Tyutyunov *et al.*,
67 2008; Papaïx *et al.*, 2011). Historical herbicide applications have been shown to drive the
68 evolution of herbicide resistance at a national scale (Hicks *et al.*, 2018). For fungicide
69 resistance, theoretical studies have revealed that combining effective MoAs over time and
70 space can delay resistance evolution (REX Consortium, 2013; van den Bosch *et al.*, 2014) and
71 that large-scale management strategies may differ from and interact with in-field strategies
72 (Parnell *et al.*, 2006). However, so far, in the absence of large-scale studies, recommendations
73 about fungicide use mostly stem from empirical studies conducted in local field trials assessing
74 the impact of different spraying strategies (Rosenzweig *et al.*, 2008; Dooley *et al.*, 2016a,b;
75 Heick *et al.*, 2017).

76

77 The aim of this study was to highlight the determinants of fungicide resistance evolution at the
78 national scale in France. We investigated the main potential drivers of evolution: (i) the
79 selection pressure effect, as assessed by regional fungicide use, (ii) the genetic drift effect,
80 which is modulated by population size (Maxwell *et al.*, 1990; Sisterson *et al.*, 2004), using yield
81 losses as a proxy and (iii) the refuge effect, including the fraction of wheat fields unsprayed
82 with fungicides over the territory (Parnell *et al.*, 2006; Tabashnik *et al.*, 2008), assessed by
83 determining the area under organic wheat. Although organic areas may not be the only fields
84 not sprayed for a given fungicide (e.g. conventional areas not using this given fungicide), they

85 represent at least a lower bound for refuges.

86

87 We focused our analysis on *Zymoseptoria tritici* (formerly *Septoria tritici* and *Mycosphaerella*
88 *graminicola* as teleomorph), an ascomycete responsible for septoria leaf blotch (STB) on
89 winter wheat. *Z. tritici* has many features facilitating the emergence of resistance: high genome
90 plasticity, a large population size, high genetic diversity, asexual and sexual reproduction, an
91 ability to disperse over large distances (Zhan & McDonald, 2004; Croll & McDonald, 2012).
92 STB is a major wheat disease that can cause yield losses of up to 50% (Ponomarenko *et al.*,
93 2011; Torriani *et al.*, 2015). In western Europe, up to 70% of all fungicide use is linked to STB
94 control (Fones & Gurr, 2015). As a result, various degrees of resistance to all authorised unisite
95 inhibitors (*i.e.* exhibiting a single molecular mode of action) have been observed in France
96 (Garnault *et al.*, 2019).

97

98 We previously published an initial analysis of the Performance trial network dataset, in which
99 phenotypes of resistance to four fungicide MoAs were monitored annually, from 2004 to 2017,
100 at about 70 sites throughout France (Garnault *et al.* 2019). We found significant differences
101 between resistance phenotypes in terms of changes in spatial distribution and/or growth rates.
102 Major differences in population structure and dynamics were highlighted between the north
103 and south of France.

104

105 We develop here an explanatory model for identifying the determinants of these regional
106 spatiotemporal heterogeneities in resistance evolution according to resistance phenotype. We
107 investigated the effect of annual fungicide use, pathogen population size and the fraction of
108 refuges, all at the regional scale (spatial units of 25 000 squared kilometers in average). The
109 use of the regional scale was encouraged by the fact that (*i*) the use of fungicide use in France
110 was well-documented at this scale, from panel surveys, (*ii*) the distribution of resistance
111 frequency data we had in France was fairly well adapted to this scale, (*iii*) we wanted to keep
112 homogeneity with works published in Garnault *et al.* (2019). Our analysis shows that the

113 change in resistance frequency can be assessed at regional scale, and that the major
114 determinant of resistance is the selection pressure exerted by fungicide applications in the
115 preceding year. This study provides empirical results for regional resistance management, at
116 a level intermediate between field and national recommendations. A sound understanding of
117 resistance evolution and of its determinants would help optimizing resistance management
118 and applying them at sound spatiotemporal scales. It should ultimately help to reduce pesticide
119 use in agrosystems.

120

121 **2 MATERIALS AND METHODS**

122 **2.1 Data description**

123 **2.1.1 Sampling of *Z. tritici* populations and estimation of resistance frequency**

124 The “Performance network” is supervised by ARVALIS-Institut du Végétal and the INRAE
125 research institute at Thiverval-Grignon. It carried out field trials on wheat throughout France
126 between 2004 and 2017 with a mean of 70 trials annually (4 to 5 trials per region and per year,
127 90% credible interval is 1 to 10). The frequency of resistant phenotypes in *Z. tritici* populations
128 sampled annually in these trials is recorded in the associated dataset (see Garnault *et al.*, 2019
129 for further information).

130 Wheat trials were carried out in a randomized block design with 3 to 4 replicates. The frequency
131 of resistant phenotypes in population were estimated by collecting bulk pycnidiospores from
132 30 to 40 upper leaves, that were randomly sampled within each plot and showed STB
133 symptoms. Cropped cultivars were predominantly STB-sensitive to promote the presence of
134 the disease, a total of 124 different wheat cultivars were cropped over the whole studied period.
135 Phenotypes were distinguished on the basis of their germination or growth on Petri dishes
136 containing discriminatory doses of fungicides, optimised on individual genotyped isolates (see
137 Leroux & Walker, 2011 and Garnault *et al.*, 2019 for more details). We then considered: (i) the
138 phenotype displaying specific qualitative resistance to strobilurins (or Qols; inhibitors of
139 respiration complex III), hereafter referred to as the StrR phenotype, (ii) the group of
140 phenotypes with moderate quantitative resistance to DMIs (sterol 14 α -demethylation

141 inhibitors), hereafter referred to as TriMR phenotypes, (iii) the group of phenotypes with a high
142 quantitative resistance to DMIs, hereafter referred to as TriHR phenotypes. The TriMR group
143 encompasses the TriR6 and TriR7-TriR8 phenotypes, which were also included in the analysis
144 (TriR6 strains were recognised on the basis of their growth on low doses of prochloraz,
145 contrasting with the lack of growth of TriR7–TriR8 strains in these conditions; Leroux & Walker,
146 2011).

147
148 Region, year, sampling date and cultivar grown were recorded for each sample. We considered
149 only populations from unsprayed plots for this study. Regions corresponded to administrative
150 spatial entities ($n= 22$) whose mean surface was 25 000 km². French regions roughly match
151 the agro-pedo-climatic heterogeneity of the national territory. The plots were sampled at two
152 time points: at “S1” in April-May, at about the Z32 wheat stage ($n=1320$, from 2006 to 2011),
153 and at “S2” in May-June, at about the Z39-Z55 wheat stage ($n=2407$, from 2004 to 2017).

154
155 In this study, we focused on the phase of resistance selection. We therefore extracted from
156 the Performance dataset the time periods during which resistance frequencies were
157 increasing, *i.e.* with estimated positive national growth rates (see Garnault *et al.*, 2019). These
158 periods were 2004 to 2012 ($n=852$, 16 regions) for the StrR phenotype, 2005 to 2011 ($n=754$,
159 16 regions) for the TriMR phenotype group, and 2010 to 2017 ($n=360$, 14 regions) for the TriHR
160 phenotype group. We also included data from 2006 to 2017 for the TriR6 and TriR7-TriR8
161 phenotypes ($n=910$ and $n=851$, respectively), for analysis of the spatial heterogeneity of their
162 frequencies. The data are summarised in Table 1.

163

164 **2.1.2 Regional fungicide use**

165 Every year, Bayer Crop Science uses field surveys to estimate the area of wheat sprayed with
166 fungicides containing anti-STB active ingredients (AIs) in each region of France. These data
167 do not include information about the dose used in the application. They only provide
168 information about the areas sprayed with the AIs concerned and the number of sprayings

169 (areas result from the multiplication of these two values). These areas are expressed in
170 deployed hectares.

171
172 We retained the most widely used AIs for each MoA (AIs accounting cumulatively for more
173 than 95% of the use of the MoA), to prevent background noise from AIs with a limited impact
174 on STB control. The model therefore included pyraclostrobin (26%), azoxystrobin (19%),
175 trifloxystrobin (15%), kresoxim-methyl (15%), fluoxastrobin (12%) and picoxystrobin (12%) for
176 Qols; and epoxiconazole (30%), prochloraz (17%), tebuconazole (13%), cyproconazole (11%),
177 prothioconazole (10%), propiconazole (7%), metconazole (7%), fluquinconazole (2%) and
178 hexaconazole (1%) for DMIs.

179
180 We took the regional heterogeneity in wheat production between regions (and, hence, in the
181 area sprayed with fungicides) into account, by dividing the number of deployed hectares by
182 the regional area under conventionally farmed wheat. The latter was calculated by subtracting
183 the area under organic wheat (see section 2.1.4) from the total area under wheat (from the
184 AGRESTE online data: agreste.agriculture.gouv.fr) for each year and region. This new variable
185 unit was named $ha_{\frac{D}{C}}$ (D for deployed and C for cropped hectares), and was proportional to the
186 mean number of times each AI was used over a cropping season in a given region. The
187 national trend and the regional heterogeneity of fungicide use expressed in $ha_{\frac{D}{C}}$ are shown for
188 DMIs and Qols in Fig. 1. Henceforth, this variable is denoted F_{itf} , with f corresponding to the
189 AI, t to the year and i to the region.

190

191 **2.1.3 Yield losses induced by STB**

192 ARVALIS-Institut du Végétal assessed annual yield loss by conducting paired plot experiments
193 throughout France with a mean of 80 trials annually (21 observations per region and per year
194 distributed among 3 to 4 trials, 90% credible interval is 9 to 48), from 2004 to 2017, in 20
195 French regions (Arvalis, 2019). In each trial, we considered modalities cropped with STB-

196 susceptible wheat cultivars, in both unsprayed plots and sprayed plots (providing maximum
197 protection against diseases). These cultivars were moderately to highly resistant to rusts in
198 order to attribute yield losses mainly to STB. Yield losses due to STB were calculated by
199 subtracting the yield in the unsprayed plot from that in the sprayed plot. Based on these data,
200 we predicted regional yield losses for each year with a linear model (fixed effects: year, region;
201 random effects: wheat cultivar, trial). The national trend and the regional heterogeneity of yield
202 losses, expressed in decitons per hectare, are shown in Fig. 1. This variable is denoted P_{it}
203 hereafter, with t corresponding to the year and i to the region.

204

205 **2.1.4 Proportion of the total area under wheat farmed organically**

206 The area under organically farmed wheat crops was recorded by AgenceBIO (the French
207 national platform for the promotion and development of organic farming) and ARVALIS-Institut
208 du Végétal. We collected regional data from 2007 onwards, and national data from 2004
209 onwards. The regional areas under organic wheat between 2004 and 2006 were assessed
210 from the observed mean proportions of the regional area under organic wheat in subsequent
211 years and from national data for 2004 to 2006. We used the regional proportion of wheat under
212 organic farming in our models. This proportion was calculated by dividing the regional area
213 under organic wheat by the total area under wheat in the same region, based on AGRESTE
214 online data. The national trend and the regional heterogeneity of the area under organic wheat,
215 expressed in hectares, are shown in Fig. 1. This variable is denoted R_{it} hereafter, with t
216 corresponding to the year and i to the region.

217

218 **2.2 Statistical modelling**

219 We modelled the change in frequency for each resistance phenotype in French populations.
220 The model took into account (i) the different phases of resistance dynamics (see below), (ii)
221 the effects of previously described potential regional determinants and finally (iii) variability due
222 to the sampling design (sampling date and wheat cultivar).

223

224 **Phases in resistance dynamics.** We distinguished three phases in resistance dynamics: “no
 225 resistance” (frequency equal to 0), “resistance selection” and “generalized resistance”
 226 (frequency equal to 100). During the “resistance selection” phase, observations were modelled
 227 with binomial random variables with a sample size of 100 (mean number of observed spores
 228 used to determine frequencies). The probabilities that a population was in the “no resistance”,
 229 “generalized resistance” or “resistance selection” phase depended on the year t . These
 230 probabilities were referred as π_{0t} , π_{100t} and $(1 - \pi_{0t} - \pi_{100t})$, respectively. Thus, Y_{itjkn} , the
 231 n^{th} frequency observed in region i , in year t , on cultivar j and at sampling date k followed a
 232 zero-and-one inflated binomial distribution (Eqn 1).

233

234 **Eqn 1**

$$235 \quad Y_{itjkn} \begin{cases} = 0 & \text{with probability } \pi_{0t} \\ \sim \mathcal{B}(100, p_{itjkn}) & \text{with probability } 1 - \pi_{0t} - \pi_{100t} \\ = 100 & \text{with probability } \pi_{100t} \end{cases}$$

236

237 **Resistance evolution.** Using a logit transformation (Eqn 2), the proportion p_{itjkn} of resistant
 238 phenotypes during resistance evolution was modelled by the regional dynamics D_{it} and the
 239 variability due to sampling design ζ_{itjkn} .

240

241 **Eqn 2**

$$242 \quad \text{logit}(p_{itjkn}) = \ln\left(\frac{p_{itjkn}}{1 - p_{itjkn}}\right) = D_{it} + \zeta_{itjkn}$$

243

244 **Regional dynamics.** The change in resistance frequencies depended on regional-scale
 245 variables: fungicide use, yield losses and areas under organic farming. The regional dynamics
 246 $D_{i(t+1)}$ in region i at year $t+1$ was obtained by adding the regional dynamics of the previous
 247 year D_{it} to the additive effects of fungicide use ϕ_{it} , yield loss ρP_{it} and wheat area under organic
 248 farming κR_{it} in year t (Eqn 3). D_{i1} is related to the logit of the initial resistance frequency in

249 region i . We kept values for the fungicide uses and areas under organic wheat raw (*i.e.* neither
250 centered nor reduced), while we centered values for the yield losses. Thus, the parameter β
251 corresponds to a continuous shift in resistance frequency in absence of fungicide and refuges
252 with an average population size. This parameter will be interpreted as an apparent fitness
253 penalty if estimated negative, and as an apparent fitness advantage otherwise.

254

255 **Eqn 3**

$$256 \quad D_{i(t+1)} = D_{it} + \beta + \phi_{it} + \rho P_{it} + \kappa R_{it} \quad \text{with } t \geq 1$$

257

258 We derived two models from Eqn 3: one in which the use of fungicides is specified for each AI
259 within MoAs, and another in which only the global use of each MoA (*i.e.* sum of AIs uses) is
260 considered.

261

262 In the first model, \mathbf{M}_{AI} , the term ϕ_{it} from Eqn 3 was defined as in Eqn 4:

263

264 **Eqn 4**

$$265 \quad \phi_{it} = \sum_{f \in \mathcal{F}} \nu_f F_{itf}$$

266

267 F_{itf} corresponds to the fungicide use for a specific AI f , in region i and year t (see section 2.1.2).

268 The set \mathcal{F} included all AIs in a specific class associated with considered resistance: Qols for
269 the StrR phenotype, DMIs for the TriR phenotypes. We assumed that fungicide use positively
270 selected resistant phenotypes over susceptible or less sensitive phenotypes. Thus, the
271 parameters associated with fungicide use were, by definition, positive (*i.e.* $\nu_f \geq 0$), except for
272 the TriR6 and TriR7-TriR8 phenotypes, which were not the most DMI-resistant phenotypes
273 over their study period.

274

275 In the second model, \mathbf{M}_{MoA} , we considered: $F_{it.} = \sum_{f \in \mathcal{F}} F_{itf}$, the regional use of a given MoA.
 276 The term ϕ_{it} from Eqn 3 was simplified and written: $\phi_{it} = \nu * F_{it.}$ This model was run only for
 277 the StrR, TriMR and TriHR phenotypes, with, as above, the constraint $\nu \geq 0$.

278

279 **Observation variability.** The variability ζ_{itkln} of observations in Eqn 2 was modelled with a
 280 mixed model (Equation 5).

281

282 Equation 5

$$283 \zeta_{itjkn} = \gamma_j + \delta_k + \varepsilon_{itjkn}$$

284

285 The parameter γ_j corresponds to the random effect of the 124 wheat cultivars (γ_j drawn from
 286 a centered Gaussian distribution with standard deviation σ_γ). The parameter δ_k is the fixed
 287 effect of sampling date k (i.e. “S1” or “S2”, see section 2.1.1, with contrast $\delta_{S2} = 0$). Finally,
 288 ε_{itjkn} is the overdispersion, modelled as a random individual effect with a mean of 0 and a
 289 standard deviation of σ .

290

291 2.3 Parameter expression

292 The explanatory variables had different units (e.g. proportion of wheat under organic farming
 293 vs. fungicide use). Moreover, the interpretation of the parameters of the zero-one-inflated
 294 logistic regression was not straightforward. We simplified the interpretation, by defining the
 295 *expected frequency difference* (EFD) for each variable and phenotype. The EFD described the
 296 frequency shift due to the mean value of this variable over a population with the mean
 297 resistance frequency (i.e. the difference between p^e , the *expected frequency*, and \bar{p} , the *mean*
 298 *frequency* of the resistance phenotype in the data). The expected frequency was computed
 299 with:

- 300 • for ν_f , the effect of fungicide use: $\text{logit}(p_{\nu_f}^e) = \text{logit}(\bar{p}) + \hat{\nu}_f \bar{F}_f$, where \bar{F}_f is the mean
 301 annual use of fungicide f over all regions, and $\hat{\nu}_f$ is the estimate of ν_f ;

- 302 • for β , the effect of constant growth: $\text{logit}(p_{\beta}^e) = \text{logit}(\bar{p}) + \hat{\beta}$;
- 303 • for ρ , the effect of yield losses due to STB: $\text{logit}(p_{\rho}^e) = \text{logit}(\bar{p}) + \hat{\rho}\bar{P}$;
- 304 • for κ , the effect of the area of wheat under organic farming: $\text{logit}(p_{\kappa}^e) = \text{logit}(\bar{p}) + \hat{\kappa}\bar{R}$;
- 305 • for δ , the effect of sampling date: $\text{logit}(p_{\delta}^e) = \text{logit}(\bar{p}) + \hat{\delta}_{T0}$;
- 306 • for σ_{γ} , the standard deviation from the cultivar effect : $\text{logit}(p_{\sigma_{\gamma}}^e) = \text{logit}(\bar{p}) \pm \hat{\sigma}_{\gamma}$.

307 The *expected frequency difference* was then calculated as $EFD = (p^e - \bar{p}) * 100$.

308

309 **2.4 Bayesian analysis**

310 Statistical analyses were performed with *R* software (R Development Core Team, 2008), in a
 311 Bayesian framework, with the *rjags* package (Plummer, 2013).

312

313 ***Prior and posterior densities.*** Non-informative prior distributions were used (Supporting
 314 Information, Eqn S1). Posterior distributions were estimated by Monte Carlo-Markov chain
 315 (MCMC) methods. Five MCMC chains were run, over 1 000 000 iterations, with a burn-in of
 316 100 000 and a thinning every 1 000 for the variable selection phase (see the following section),
 317 followed by 500 000 iterations with a burn-in of 50 000 and a thinning every 500 for the final
 318 parameter estimation. Convergence was assessed with the Gelman and Rubin \hat{R} statistic
 319 (Gelman *et al.*, 2004). Credible intervals of the highest posterior density were calculated from
 320 posterior densities with the *HDI* package (Dezeure *et al.*, 2015). Parameter estimates were
 321 considered significant at the 5% level (or the 2.5% or 0.1% level), if their 95% credible interval
 322 (97.5% and 99.9%, respectively) did not contain the 0 value.

323

324 ***Variable selection.*** We used a selection procedure to identify the relevant variables in each
 325 model for each resistance phenotype. We used a method based on indicator variables (Kuo &
 326 Mallick, 1998), in which each predictor was multiplied by a dummy variable with a prior
 327 distribution corresponding to a Bernoulli distribution with parameter $p = 0.5$. A predictor was
 328 retained in the model if the posterior expectation of its indicator variable was greater than 0.75

329 (thus, greater than its prior expectation of 0.5).

330

331 **Predictive check.** We assessed the fit of the model to the data by posterior predictive checks
332 (Gelman *et al.*, 2004). Replicated data (y_{itjkn}^{rep}) generated during the MCMC algorithm from the
333 model posterior densities were compared to observed data (y_{itjkn}). The mean value of
334 $PP_{itjkn}^{check} = P(y_{itjkn} - y_{itjkn}^{rep} < 0 | Y)$, where Y is the vector of observations, was calculated and
335 denoted PP_{check} . This value indicated the goodness of fit of the model, with a good fit
336 corresponding at $PP_{check} = 0.5$, *i.e.* with equal probabilities of over- and under-estimation

337

338 **Variable weight.** We assessed the influence of each explanatory variable θ by calculating its
339 weight (W_θ). The weight W_θ was defined as the ratio of $RSS_{full-\theta}$ to RSS_{full} , where RSS_{full} and
340 $RSS_{full-\theta}$ are the residual sums of squares of the full model (*i.e.* including all the explanatory
341 variables selected by the variable selection procedure) and of this same model but without the
342 explanatory variable θ , respectively. RSS should be minimal for the full model, so removing an
343 explanatory variable should increase RSS : the more information θ contributes, the greater the
344 increase in RSS and the higher the value of W_θ . Conversely, if the information provided by θ is
345 negligible, RSS is unaffected and W_θ is minimal (*i.e.* close to 1). In the result tables, we have
346 calculated the relative weights by dividing individual variable weights by the sum of the weights
347 of all variables.

348

349 **Model comparison.** For comparison of the M_{AI} and M_{MoA} models, we calculated the deviance
350 information criterion, DIC (Plummer, 2013), and the coefficient of determination, R^2 .

351

352 **Predicted data.** We computed predictions of the resistance frequencies for each phenotype
353 for a given region l , and a given year T (Equation 6).

354

355 **Equation 6**

356
$$\hat{Y} = [1 - (\hat{\pi}_{0T} + \hat{\pi}_{100T})] * \text{logit}^{-1}[\hat{D}_{I1} + \hat{\beta}(T - 1) + \sum_{t=1}^{T-1}(\hat{\rho}P_{It} + \hat{\kappa}R_{It} + \sum_{f \in \mathcal{F}} \hat{\nu}_f F_{Itf})] + \hat{\pi}_{100T}$$

357

358 where parameter-hat are parameter estimates (*i.e.* their posterior mean). We therefore built
359 maps of resistance status, for known initial frequencies, use of fungicides, yield losses and
360 areas under organic wheat, from year 1 to year $T-1$ in region I . \hat{Y} are then multiplied by 100 to
361 recover the initial frequency scale. These predicted data included the effect of inflation
362 parameters $\hat{\pi}_{0T}$ and $\hat{\pi}_{100T}$.

363

364 **3 RESULTS**

365 **3.1 Overview of model fits**

366 The convergence of the MCMC chain was satisfactory for all models (*i.e.* the Gelman and
367 Rubin indicator \hat{R} was below 1.1 for all parameters, in all models) and model fit was good
368 (PP_{check} always between 0.498 and 0.511).

369

370 After the selection procedure, no effect was retained for the following models: M_{AI} for the TriHR
371 resistance phenotype, and M_{MOA} for the TriMR and the TriHR groups of resistance phenotypes.
372 Thus, the effects of fungicide use, yield losses, and areas of wheat under organic farming were
373 not significant in these models. The only remaining parameter was the growth constant
374 (already studied in Garnault *et al.*, 2019). As all estimates of the parameters of interest were
375 equal to 0, we do not discuss the results of these models, and they do not appear in the result
376 tables.

377

378 Finally, with Spearman's method, a few significant correlations were found between some AI
379 uses (F_{itf}) in model inputs, but no significant correlation between estimates ($\hat{\nu}_f$) was found in
380 model outputs (Supporting Information, Fig. S1).

381

382 **3.2 Ranking of variable weight**

383 Regional fungicide use appeared to be the major factor driving resistance evolution. In the M_{AI}
384 models, which explicitly considered each AI, regional fungicide uses systematically had the
385 highest relative weight. For the StrR and TriMR phenotypes, it accounted for 87.4% and 72.6%,
386 respectively. It accounted for 53.1% and 43.3% for the TriR6 and TriR7-TriR8 phenotypes,
387 respectively (Table 2). For the M_{MoA} models, fungicide use, considered as the sum of AI uses
388 within the same MoA, was also the major determinant of the StrR phenotype (associated with
389 qualitative resistance to Qols), accounting for 79.4% of the sum of variable weights (Table 3).
390 For the TriMR and TriHR groups of phenotypes, no explanatory variables were selected for
391 the M_{MoA} models.

392

393 The growth constant was also a major parameter, albeit to a lesser extent. In M_{AI} models, the
394 weight of the growth constant was lower than that of regional fungicide use by a factor of 5.7
395 times for TriMR phenotypes, 1.32 for the TriR6 phenotype, and 1.08 for the TriR7-TriR8
396 phenotypes (Table 2). For the StrR phenotype, the growth constant ranked third, with a weight
397 lower than that of fungicide use by a factor of almost 20, for both models (Tables 2 and 3).

398

399 The proportion of the area under wheat farmed organically had a high relative weight for the
400 StrR and TriMR phenotypes, but was not selected for the TriR6 and TriR7-TriR8 phenotypes.
401 For the StrR phenotype, its effect was ranked second on the basis of relative weight, at 14.6%
402 and 6.8% in the M_{MoA} and the M_{AI} models, respectively (Tables 2 and 3). For TriMR phenotypes,
403 the relative weight of the wheat area under organic farming was about 13.3%, a value very
404 similar to that for the growth constant (Table 2). Yield loss was systematically excluded during
405 the selection procedure, for all models and all phenotypes.

406 Sampling data and wheat cultivar, variables reflecting local variability in trials, had only a low
407 relative weight in models, with values always below 5%, except for the wheat cultivar variable
408 for the TriR7-TriR8 phenotype, for which the value was 13.4% (Table 2).

409

410 3.3 Effect of variables at the regional scale

411 3.3.1 Regional fungicide use

412 For the StrR phenotype, in the M_{MoA} model, the effect of the overall use of QoI fungicides was
413 highly significant ($\nu = 1.07$, $P < 0.001$) and the expected frequency difference (EFD) was
414 estimated at 6.64%. Thus, an average use of QoIs would have led to an increase of 6.64
415 frequency point on an average population that would already be composed by 76% of StrR
416 phenotype (Table 3).

417
418 In the M_{AI} model, two fungicides from the six QoI AIs were selected: kresoxim-methyl and
419 pyraclostrobin. Their EFDs were similar: 4.26% ($\nu = 0.7$, $P < 0.001$) and 3.29% ($\nu = 0.5$, $P <$
420 0.025), respectively (Table 2). The M_{MoA} and M_{AI} models also had similar adequacies to data,
421 according to their DIC (3480.2 and 3468.8 respectively) and R^2 values (0.82 and 0.81,
422 respectively).

423
424 For TriMR phenotypes, the effect of DMI use was estimated only in the M_{AI} model, as no
425 explanatory variable was selected in the M_{MoA} model. One AI of the nine DMI fungicides was
426 selected: epoxiconazole ($\nu = 0.56$, $P < 0.025$) with an estimated positive EFD of 4.82% (Table
427 2).

428
429 As the TriR6 and TriR7-TriR8 phenotypes were not the most resistant to DMI over the study
430 period, the effect of fungicide use was not constrained to be null or positive, and was estimated
431 only in the M_{AI} model. For the TriR6 phenotype, three AIs from the nine DMI fungicides were
432 selected: prochloraz, with a positive EFD of 4.73% ($\nu = 0.85$, $P < 0.001$), propiconazole with a
433 negative EFD of -2.48% ($\nu = -0.43$, $P < 0.025$) and tebuconazole with a negative EFD of -
434 8.38% ($\nu = -1.04$, $P < 0.001$). Thus, prochloraz use increased the frequency of TriR6, whereas
435 tebuconazole counterselected TriR6 strains. For the TriR7-TriR8 phenotype, three AIs from
436 the nine DMI fungicides were selected: cyproconazole and tebuconazole, with positive EFDs
437 of 2.6% ($\nu = 0.58$, $P < 0.025$) at 2.38% ($\nu = 0.44$, $P < 0.05$), respectively, and prochloraz, with

438 a negative EFD of -3.99% ($v = -1.17$, $P < 0.001$). Prochloraz and tebuconazole clearly had
439 opposite selection effects on TriR6 and TriR7-TriR8 phenotypes.

440

441 **3.3.2 Growth constant**

442 The growth constant EFD quantified the change in resistance in the absence of regional
443 fungicide use and unsprayed refuges, for a mean potential yield loss. It therefore represented
444 the relative apparent fitness (referred to hereafter simply as fitness) of the resistant phenotype
445 considered (*i.e.* how much faster the resistant phenotype would grow compared to the rest of
446 the population (Hartl & Clark, 1997) in a year without fungicide treatment). A negative growth
447 constant indicates a fitness cost, whereas a positive growth constant indicates a fitness gain.
448 For the StrR phenotype, the growth constant provided an indication of the fitness of the
449 resistant strains relative to the sensitive strains. The estimated fitness costs for this phenotype
450 were similar in the M_{AI} (-3.74%; $\beta = -0.2$, $P < 0.05$; Table 2) and M_{MoA} (-3.23%; $\beta = -0.17$, not
451 significant; Table 3) models. DMIs selected a large diversity of phenotypes (TriLR, TriMR and
452 TriHR groups), and no sensitive strains were detected during the study period. For TriMR
453 phenotypes, the model was estimated with data from 2007 to 2011, when the frequency of the
454 TriHR phenotype was still negligible (Fig. 1). Thus, the growth constant for TriMR phenotypes
455 mostly compared their fitness with that of TriLR phenotypes. It was estimated at -3.94%, of
456 borderline significance ($\beta = -0.2$, $P < 0.1$), and was associated with a relative weight of 12.7%
457 (Table 2). The TriMR group included the TriR6 and TriR7-TriR8 phenotypes. For the TriR6 and
458 TriR7-TriR8 strains, the model was estimated with data from 2006 to 2017. However, the TriHR
459 phenotype has been non-negligible since 2014 (Figure 1). Thus, the growth constant for TriR6
460 strains compared their fitness with that of all the other phenotypes in the population: TriLR,
461 TriR7-TriR8 and TriHR. The TriR6 growth constant was estimated at 3.58% ($\beta = 0.15$, $P <$
462 0.025 ; Table 2). This result may reflect a balance between a fitness cost of TriR6 relative to
463 TriLR and TriR7-TriR8 strains, and a fitness gain relative to the TriHR phenotype. This rationale
464 also applies to the apparent fitness gain of TriR7-TriR8, estimated at +1.63% ($\beta = 0.09$, $P <$
465 0.1 ; Table 2).

466

467 **3.3.3 Wheat area under organic farming**

468 The proportion of the area under wheat management by organic farming methods increased
469 resistance frequency, with an EFD estimated at 3.95% and 3.26% in the M_{MoA} and M_{AI} models,
470 respectively, for the StrR phenotype ($\kappa = 0.78$ and 0.64 , $P < 0.001$; Tables 2 and 3), and at
471 2.52% for the TriMR phenotype in the M_{AI} model ($\kappa = 0.37$, $P < 0.001$; Table 2). This variable
472 was not selected for the TriR6 and TriR7-TriR8 phenotypes.

473

474 **3.3.4 Yield losses**

475 The selection procedure did not retain the yield loss variable in any of the models.

476

477 **3.4 Prediction maps**

478 We mapped the predicted resistance frequencies for each year and for all phenotypes. Our
479 maps predicting StrR phenotype dynamics were mostly based on regional QoI fungicide use
480 and, to a lesser extent, the area under organic wheat, as explanatory variables (Fig. 2).
481 Predictions mimicked the spatial propagation from the north to the south of France observed
482 between 2004 and 2011 (as described by Garnault *et al.*, 2019). Based on regional DMI use,
483 our model accurately predicted (Fig. 3) the observed spatial partitioning of TriR6 strains, which
484 were found mostly in the north east of France, and TriR7-TriR8 phenotypes, which were mostly
485 localised in the south west (as described by Garnault *et al.*, 2019).

486

487 **4 DISCUSSION**

488 In this study, we developed a model for identifying the determinants of fungicide resistance
489 evolution at the regional scale. The candidate explanatory variables were regional fungicide
490 use, pathogen population size (approximated by potential yield losses) and the fraction of
491 refuges (approximated by the fraction of fields under organic wheat in a region). We analysed
492 resistance frequencies in *Z. tritici* populations on winter wheat. Frequencies were monitored
493 by the Performance trial network over the national territory of French (2004–2017; ~70

494 locations each year). We studied resistance against fungicides with two modes of action: QoIs,
495 associated with qualitative resistance (StrR phenotype), and DMIs, associated with
496 quantitative resistance (a continuum of multiple resistance phenotypes forming three main
497 groups: TriLR, TriMR and TriHR, with low, medium and high levels of resistance to DMIs,
498 respectively). The TriMR phenotypes encompassed two phenotypes: TriR6 and TriR7-TriR8.

499

500 ***The regional use of fungicides is the main driver of resistance evolution at the plot scale***

501 We demonstrated that regional fungicide use was a major determinant of the evolution of
502 resistance. Fungicide use data at regional level, without taking into account the use of
503 particular fungicides in particular fields, was sufficiently informative to explain resistance
504 dynamics. Fungicide selection is, therefore, a large-scale process, and an understanding of
505 the evolution of fungicide resistance requires consideration of the regional use of these
506 compounds.

507

508 For qualitative resistance (e.g. StrR phenotype), the global use of a MoA (*i.e.* summed uses of
509 the AIs of this MoA), can be considered as a good predictor of resistance evolution and
510 distinguishing the effects of individual AIs within the MoA did not improve the fit of the model.
511 By contrast, for quantitative resistance, it appeared to be necessary to consider each AI within
512 the MoA separately, as they may select for different phenotypes (*e.g.* antagonist effects of
513 prochloraz, tebuconazole on TriR phenotypes).

514

515 The AIs selected by the model were consistent with the history of fungicide use and/or with the
516 patterns of cross-resistance described for some phenotypes. For instance, kresoxim-methyl
517 was one of the first QoIs authorised in France (commercialised in 1997, www.ephy.anses.fr)
518 and was selected to explain the evolution of StrR strains, whereas more recent QoIs were not.
519 The fungicides most used over the study period (*i.e.* epoxiconazole for DMIs, pyraclostrobin
520 and kresoxim-methyl for QoIs; Supporting Information, Fig. S2-S3), were also selected in
521 models. The large-scale effect of AIs was also linked to the cross-resistance pattern of the

522 phenotypes. The estimated selection effect of epoxiconazole was consistent with resistance
523 factors (RFs) of TriMR phenotypes to epoxiconazole, being higher than those of TriLR
524 phenotypes which were the second most frequent resistance phenotype during this period
525 (RFs described in Supporting Information, Table S1 from Leroux & Walker, 2011). The
526 selection for TriR6 and counterselection for TriR7-TriR8 strains induced by the prochloraz were
527 consistent with RFs (6.7 and <1.5, respectively), as well as the effect of counterselection for
528 TriR6 and selection for TriR7-TriR8 strains by tebuconazole (RF=74 and 91, respectively for
529 TriR6 and TriR8 strains, TriR7 strains being unfrequent (Huf *et al.*, 2018)). Propiconazole and
530 cyproconazole were often used together as a mixture, so were strongly correlated (Supporting
531 Information, Fig. S1). The effects of counterselection for TriR6 strains by propiconazole and of
532 selection for TriR7-TriR8 by cyproconazole may indicate that their mixture globally promoted
533 the selection of TriR7-TriR8 strains over TriR6 strains. Again, this is consistent with RFs to
534 propiconazole (35 and 54, respectively for TriR6 and TriR8 strains) and to cyproconazole (11
535 and 13, respectively). These findings also confirm that the laboratory characterisation of strains
536 can be good predictor of resistance evolution in the field, if properly used, as reported by Blake
537 *et al.* (2018).

538

539 Our findings highlight the importance of defining precise resistance profile phenotypes. Indeed,
540 the model fitted slightly better the frequencies on more homogeneous phenotypes ($R^2 = 0.48$
541 for TriMR vs. 0.56 for TriR6 and TriR7-TriR8 phenotypes). In addition, no significant effect of
542 the different DMIs was found for the TriHR phenotype group. Indeed, TriHR strains encompass
543 multiple heterogeneous phenotypes, resulting from combinations of target alteration, target
544 overexpression and enhanced efflux, as resistance mechanisms (Leroux & Walker, 2011; Huf
545 *et al.*, 2018). The tremendous diversity and redundancy of phenotypes observed in the field,
546 especially for TriHR strains, making it possible to classify strains only approximately. There is
547 therefore a need to develop molecular tools for quantifying genotypes rather than phenotypes.
548 Multi-trait high-throughput genotyping provides a more accurate resistance frequency, and
549 should ultimately lead to improvements in our ability to predict resistance evolution.

550

551 ***The proportion of the wheat area under organic farming may be still too limited to***
552 ***mitigate the evolution of resistance via a refuge effect***

553 Wheat areas managed under organic farming systems are not treated with synthetic
554 fungicides. They represent a lower bound of the surfaces that are not sprayed for a given MoA,
555 as some conventional field may also be not sprayed with the considered MoA (which is more
556 true for Qols and SDHIs, than for DMIs as they are quasi-systematic fungicides in conventional
557 wheat farming). Organic areas could act as refuges for “wild” susceptible or less resistant
558 individuals, which may reproduce, delaying the evolution of resistance by a dilution effect in
559 mobile species (Gould, 2000). But refuges may also provide a heterogeneous environment,
560 promoting sink-source dynamics: selection-free wheat areas acting as a source of susceptible
561 strains that migrate towards areas farmed conventionally. These opposite effects have been
562 described in studies of the resistance to transgenic crops expressing *Bacillus thuringiensis* (*Bt*)
563 toxins, where non-*Bt* crops may act as refuges diluting the selection (Huang *et al.*, 2011), or
564 promoting the evolution of resistance (Caprio, 2001).

565

566 The refuge effect for *Z. tritici* is, theoretically, weak due to the haploid nature of this organism
567 (Shaw, 2009), but this has never been studied experimentally. We did not validate the
568 beneficial effect of refuges, approximated by the area under wheat farmed organically. On the
569 contrary, we estimated that the selection of StrR and TriMR phenotypes would increase with
570 wheat areas under organic farming. However, the weight of this explanatory variable remained
571 much lower than that for regional fungicide use. This effect was not found significant for the
572 other phenotypes.

573

574 According to Huang *et al.* (2011), three conditions must be satisfied for refuge strategies to be
575 successful: selection at “high dose”, a very low initial frequency of resistance, and sufficient
576 refuge areas located nearby. In our study, initial frequencies of resistance (*i.e.* at the very
577 beginning of our study) were already quite high (up to 85% in some regions, Fig. 1). In addition,

578 the area under organic farming may still be too small within the landscape (generally less than
579 1% until 2010). Nevertheless, since the 2010s, the proportion of wheat under organic farming
580 has steadily increased (Fig. 1). The effect of the area under organic wheat may become
581 detectable in those areas, and should be investigated further, particularly for emerging
582 resistance phenotypes.

583

584 ***The growth constant reveals a fitness penalty of resistant phenotypes***

585 The growth constant represents the evolution of resistant phenotypes in the absence of
586 fungicide treatment. It represents the apparent fitness of the phenotype relative to that of the
587 susceptible phenotype (or other resistant phenotypes, in the case of quantitative resistance).
588 The term “apparent” is used because this quantification takes place in current crop conditions.
589 The fitness cost of resistance to drugs is known to be a key parameter driving effective anti-
590 resistance strategies (Andersson & Hughes, 2010; Melnyk *et al.*, 2015; Mikaberidze &
591 McDonald, 2015). If there is no fitness cost, regardless of the strategy used, it will always result
592 in the irreversible selection of resistance. However, it remains difficult to infer the global fitness
593 cost of a mutation throughout the entire life cycle of a pathogen (Hollomon, 2015).

594

595 We inferred an apparent relative fitness penalty for StrR phenotypes, resulting in an annual
596 decrease of 3.74%. This was consistent with the fitness cost described by Hagerty & Mundt
597 (2016) using virulence comparison tests. The impact of *cyp51* alterations, leading to TriR
598 phenotypes, on fitness is often evoked to explain the evolution of azole resistance in *Z. tritici*
599 populations (Cools *et al.*, 2013; Blake *et al.*, 2018) but it has not been quantified as yet. Our
600 model is consistent with these assumptions as it also inferred an apparent fitness penalty for
601 the TriMR group (-3.94% per year). Decreases in the use of QoI and DMI fungicides, and/or
602 the implementation of strategies favouring the expression of a resistance cost, may help to
603 slow the evolution of resistance. From our estimates, we can extrapolate a theoretical
604 equilibrium between resistance cost and selection, which could have led to a null growth of the
605 StrR and TriMR phenotypes by reducing by 50% the use of QoIs and 18% for DMIs.

606

607 For the TriR6 and TriR7-TriR8 phenotypes, the growth constant is an integrative value, as
608 these phenotypes were studied alongside more susceptible phenotypes (TriLR before 2010)
609 and more resistant phenotypes (TriHR after 2010). The positive growth constants we estimated
610 for these two phenotypes may indicate a fitness benefit of TriMR relative to TriLR phenotypes
611 and/or fitness cost of TriHR (*i.e.* also fitness benefit of TriMR relative to TriHR phenotypes).
612 Our model could be extended to determine the relative fitnesses of each phenotype in cases
613 of quantitative resistance. The global informative indicator provided by our model could be
614 used to guide the design of optimal large-scale fungicide deployment strategies.

615

616 ***The yield losses caused by STB do not affect resistance evolution***

617 Population size, a major parameter in population adaptation (Good *et al.*, 2012), is generally
618 positively correlated with resistance evolution (Weber & Diggins, 1990; Anderson, 2005; zur
619 Wiesch *et al.*, 2011). Indeed, a large population size increases the number of mutants
620 generated and decreases genetic drift (Linde *et al.*, 2002). We inferred this effect using
621 potential yield losses caused by STB as a proxy, but no significant effect was detected for any
622 resistance phenotype. Population size may not be limiting for resistance evolution in *Z. tritici* ,
623 since even in low abundance year the number of individuals remains colossal (Zhan *et al.*,
624 2001; Mikaberidze *et al.*, 2017), particularly when considering large scales. Population size
625 may not be described accurately enough as our proxy variable also depends on the timing of
626 infection (Shaw & Royle, 1993) and on stubble management (McDonald & Mundt, 2016).

627

628 ***Predicting resistance evolution over years***

629 Prediction maps can be computed from our model, using only the initial regional frequencies
630 of the resistant phenotypes, the history of fungicide use (between the initial year and the year
631 to be predicted) and the history of area under organic farming. Predictions for the StrR
632 phenotype from 2004 to 2011 highlighted the same colonisation front structure from the North
633 to the South of France (Fig. 2) as reported by Garnault *et al.* (2019) albeit the regions are

634 assumed to be fully independent. Besides, the way StrR propagated from North to South, may
635 indicates that integrating regional interdependencies in the model would improve its
636 performance. However, this would require a much more complex model and more data to
637 estimate these flux. Predictions for the TriR6 and TriR7-TriR8 phenotypes also yielded stable
638 spatial distributions between North-East and South-West France (Fig. 3), as previously
639 observed in Garnault *et al.* (2019).

640 Further analysis will be required to assess the prediction quality of the model. Nevertheless,
641 this finding supports the global validity of our model and paves the way for an original approach
642 to predicting resistance evolution in a heterogeneous landscape.

643

644 **Conclusion**

645 We developed a model of resistance dynamics, which identified regional fungicide use as the
646 major determinant of fungicide resistance evolution, and the area under organic farming as a
647 much weaker explanatory variable. We estimated the apparent relative fitness of resistant
648 phenotypes, a key parameter for the development of sustainable resistance management
649 strategies. We also identified active ingredients which use drove resistance evolution.

650 In conclusion, we showed here that the determination of resistance evolution occurs at a large
651 scale and demonstrating that concerted collective action is required, to reinforce individual
652 initiatives to tackle resistances effectively.

653

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664

665 **6 AUTHOR CONTRIBUTIONS**

666 GC was responsible for Performance network administration and supervision and for
667 establishing the dataset. PL, ASW and CD carried out laboratory manipulations on the network
668 samples to determine resistance frequencies in *Z. tritici* populations. FC and OD were involved
669 in the development of the methodology and provided theoretical support for statistical aspects
670 for data analyses. MG handled data, coded the model, performed statistical analyses and
671 generated most of the results (figures, maps and tables) during his PhD work. The article was
672 written by MG, FC, ASW and OD.

673

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817

Phenotype	First year observed	Number of years observed	Number of regions monitored	Number of regions monitored yearly	Number of observations
StrR	2004	9	16	9	852
TriMR	2005	7	16	13	754
Total					
TriR6	2006	12	16	6	910
TriR7-TriR8	2006	12	16	6	851
TriHR	2010	8	14	6	360
Total					3727

818 **Table 1** Data from the Performance database used for the statistical analysis.

819 Each observation corresponds to a resistance frequency measurement. The first column
820 indicates the year at which frequencies of the given phenotype has been first monitored in the
821 Performance network. The second column corresponds to the total number of years that the
822 given phenotype has been monitored, including the first year. The third column gives the total
823 number of French regions in which the given phenotype has been sampled. The fourth column
824 is the same as the previous one, but only with regions with at least one observation each year.
825 All regions were monitored for at least 80% of the years studied. The last column sums up the
826 total number of data points for each phenotype.

827

	Phenotype resistant to Qols			Phenotype resistant to DMIs									
	StrR (2004-2012)				TriMR (≤ 2011)			TriR6 (2006-2017)			TriR7-TriR8 (2006-2017)		
Variable	Estimate ± 1 SD	EFD ($\bar{p}=76\%$)	Relative weight		Estimate ± 1 SD	EFD ($\bar{p}=75\%$)	Relative weight	Estimate ± 1 SD	EFD ($\bar{p}=54\%$)	Relative weight	Estimate ± 1 SD	EFD ($\bar{p}=21\%$)	Relative weight
Growth constant (β)	-0.2 * ± 0.09	-3.74	0.039		-0.2 · ± 0.11	-3.94	0.127	0.15 ** ± 0.05	3.58	0.402	0.09 · ± 0.05	1.63	0.402
Fungicide use (v_f)			0.874				0.726			0.531			0.433
Qol Kresoxim- methyl	0.7 *** ± 0.14	4.26		DMI Cyproconazole	0			0			0.58 ** ± 0.2	2.6	
Pyraclostrobin	0.5 ** ± 0.17	3.29		Epoxiconazole	0.56 ** ± 0.18	4.82		0			0		
Azoxystrobin	0			Prochloraz	0			0.85 *** ± 0.16	4.73		-1.17 *** ± 0.17	-3.99	
Fluoxastrobin	0			Propiconazole	0			-0.43 ** ± 0.14	-2.48		0		
Picoxystrobin	0			Tebuconazole	0			-1.04 *** ± 0.19	-8.38		0.44 * ± 0.21	2.38	
Trifloxystrobin	0			Fluquinconazole	0			0			0		

			Hexaconazole	0		X		X				
			Metconazole	0		0		0				
			Prothioconazole	0		0		0				
Yield losses (ρ)	0		0	0		0	0	0	0	0		
Area under organic farming (κ)	0.64 *** ± 0.14	3.26	0.068	0.37 *** ± 0.01	2.52	0.133	0	0	0	0		
Wheat cultivar (σ_Y)	0.26 ** ± 0.08	± 4.68	0.005	0.14 * ± 0.06	± 2.64	0.006	0.21 ** ± 0.07	± 5.19	0.024	0.37 *** ± 0.09	± 6.18	0.134
Sampling date (δ_{S1})	-0.41 *** ± 0.1	-8.16	0.014	-0.17 ** ± 0.06	-3.34	0.008	-0.33 *** ± 0.08	-8.23	0.043	0.15 · ± 0.08	2.62	0.031

828 **Table 2** Estimates from the M_{AI} model for the StrR, TriMR, TriR6 and TriR7-TriR8 resistance phenotypes.

829 For each phenotype, the three subcolumns show parameter estimates (*i.e.* posterior mean) and their variability (*i.e.* posterior standard deviation),
830 expected frequency difference (EFD, *i.e.* the change in frequency due to the mean value of the variable on a reference population at frequency
831 \bar{p}), and relative weights (*i.e.* the contribution of the variable to data variability). The significance thresholds are 0.1, 0.05, 0.025 and 0.001 denoted
832 by “.”, “*”, “**” and “***”, respectively. Rows represent the different parameters of the models, ordered as follows: growth constant, regional scale
833 explanatory variables (fungicide use, yield losses and area of wheat under organic farming) and local variation factors (wheat cultivar and sampling
834 date). “0” means that the parameter was not selected during variable selection. “X” means that the variable was not considered in the model for
835 the given phenotype. The results for the TriHR phenotypes are not shown as no explanatory variables were retained by the selection procedure.

	StrR (2004-2012)		
Variable	Estimate ± 1 SD	EFD (\bar{p} =76%)	Relative weight
Growth constant (β)	-0.17 ± 0.13	-3.23	0.041
Fungicide use (ν)	1.07 *** ± 0.26	6.64	0.794
Yield losses (ρ)	0		0
Area under organic farming (κ)	0.78 *** ± 0.16	3.95	0.146
Wheat cultivar (σ_γ)	0.31 ** ± 0.09	±5.57	0.009
Sampling date (δ_{S1})	-0.35 *** ± 0.1	-6.87	0.01

837 **Table 3** Estimates from the M_{MoA} model for the StrR phenotype.

838 The three subcolumns show the parameter estimates (*i.e.* posterior mean) and their variability
839 (*i.e.* posterior standard deviation), expected frequency difference (EFD, *i.e.* the change in
840 frequency due to the mean value of the variable on a reference population at frequency \bar{p}),
841 and relative weights (*i.e.* the contribution of the variable to data variability). The significance
842 thresholds are 0.1, 0.05, 0.025 and 0.001 denoted by “.”, “*”, “***” and “****”, respectively. Rows
843 represent the different parameters of models, ordered as follows: growth constant, regional
844 scale explanatory variables (fungicide use, yield losses and area of wheat under organic
845 farming) and local variation factors (wheat cultivar and sampling date). “0” means that the
846 parameter was not selected during variable selection. The results for TriMR and TriHR
847 resistance phenotypes are not shown as no explanatory variable was retained by the selection

848 procedure.

849

850 **Fig. 1** Changes in resistance frequencies (left) and explanatory variables (right, top to bottom,
851 fungicide use, yield losses and area of wheat under organic farming).
852 Bold lines: mean regional values. Shaded areas: quantiles of regional values (*i.e.* regional
853 variability), 25% and 75% (dark grey), 2.5% and 97.5% (light grey). Dotted lines: regional
854 minimum and maximum values. Fungicide use is expressed in $ha_D \frac{c}{c}$ corresponding to the mean
855 number of times each mode of action was used for spraying over a cropping season,
856 regardless of the dose used. Yield losses are expressed in decitons (quintals) per hectare.
857

858 **Fig. 2** Maps of observed and predicted frequencies of the StrR resistance phenotype from
859 2004 to 2011.

860 Real observations are represented by dots. The colour within the dot indicates the observed
861 frequency in trials. The background map color shows the regional prediction from the M_{AI}
862 model.

863

864 **Fig. 3** Maps of observed and predicted frequencies of TriR6 and TriR7-TriR8 resistance
865 phenotypes in 2008.
866 Real observations are represented by dots. The colour within the dot indicates the observed
867 frequency in trials. The background map colour shows the regional prediction from the M_{AI}
868 model. Top: TriR6. Bottom: TriR7-TriR8.