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# SPATIOTEMPORAL DYNAMICS OF FUNGICIDE RESISTANCE IN THE WHEAT PATHOGEN *ZYMOSEPTORIA TRITICI* IN FRANCE

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## ABSTRACT

**BACKGROUND: Management of pesticide resistance is a major issue in modern agricultural systems, particularly in the context of the broader challenge of reducing pesticide use. However, such management must be adapted to resistance dynamics, which remains challenging to predict due to its dependence on many biological traits of pests, interactions with the environment and pesticide use. We retrospectively studied the evolution of reported resistances to four modes of action (benzimidazoles, quinone outside inhibitors, sterol demethylation inhibitors and succinate dehydrogenase inhibitors); in French populations of the wheat pathogen *Zymoseptoria tritici*.**

**RESULTS: We used statistical models to analyse the *Performance* trial network dataset (2004-2017; ~70 locations in France yearly). They highlighted contrasting behaviours between phenotypes, such as: (*i*) stable spatial distributions and colonization front structures over time; (*ii*) different frequency growth rates at the national scale and between regions.**

**CONCLUSION: We provide here a quantitative description of the spatiotemporal patterns of resistance evolution, for fungicides with several modes of action. Moreover, we highlight some unexpected resistance dynamics in France, with major differences between the north and south. This complex pattern of resistance evolution in French populations is consistent with previous descriptions of dynamics at the European scale. These results should make it easier to anticipate the evolution locally and to improve the management of resistances.**

# 1 INTRODUCTION

In modern agricultural systems, pesticides, or plant protection products (PPPs), make a major contribution to the maintenance of crop productivity, by reducing the impact of pests on yields and quality. However, human health and environment protection issues have driven national and European authorities to adopt policies limiting pesticide registration and use, as in the Ecophyto II plan in France and the 2009/128/CE directive (www.agriculture.gouv.fr). The emergence of resistance is an additional unintended issue of the widespread use of PPPs in agriculture[1]. Pesticide resistance is the intrinsic, inheritable ability of some pest genotypes to survive pesticide concentrations that kill or inhibit the development of sensitive genotypes of the same species. It results from the adaptive evolution of pest populations under pesticide pressure, leading to the selection and increase in frequency of the least sensitive genotypes[2,3]. This phenomenon may lead to practical resistance, defined as a decrease in the efficacy of a pesticide in the field. In practice, field resistance may lead to an increase in the number of sprays and the dose of pesticide used to maintain the same level of protection, with a greater toxicological and ecotoxicological impact. The development of resistance management strategies is clearly a major challenge, if we are to adhere to a long-term vision of more environmentally friendly agriculture and to avoid the dilemma of "the Tragedy of the Commons" in which multiple individuals acting independently and exclusively in their own interests deplete a limited shared resource, even though this outcome is clearly in no-one's long-term interest[4]. For the effective management of pesticide resistance, we therefore need to consider the ability of particular management strategies to limit the selection of resistance to a given pesticide in a given pest, and the factors determining sustainability. Resistance dynamics results from interactions between selection pressures, and biological and agronomic system factors promoting resistance[5]. A better understanding of these interactions is a prerequisite for the identification of the most suitable strategies for a given situation. Unfortunately, quantitative information about resistance dynamics is often limited, because the collection of such information requires long-term comprehensive monitoring over large territories, funding and stakeholder coordination.

In this context, we focused on the example of fungicide resistance in *Zymoseptoria tritici* (formerly *Septoria tritici*; teleomorph, *Mycosphaerella graminicola*). *Z. tritici* is an ascomycete that causes *Septoria tritici* blotch (STB) on winter wheat. During the cropping season, *Z. tritici* mostly disseminates over short distances by producing pycnidiospores (generated by asexual reproduction and dispersed by splashing on wheat plants), but it can also disseminate over larger distances through the production of ascospores (produced by sexual reproduction, predominantly on crop debris during the short period between crops, but also on the first senescent leaves of wheat during the cropping season[6]). *Z. tritici* is a relevant model system for addressing the issues raised above, for a number of reasons. First, it is a pathogen of considerable agronomic relevance. STB is the most damaging disease of wheat in Europe, causing yield losses of up to 50%[7–9]. Second, STB is mostly controlled by fungicides in Western Europe, with up to 70% of all fungicide use linked to STB control in the EU[9]. Third, *Z. tritici* presents biological traits that facilitate its adaptation to selective pressures, particularly those exerted by fungicides: a large population size, considerable genetic diversity, two modes of reproduction and an ability to disperse over long distances[10]. Finally, this pathogen has developed diverse resistance mechanisms, resulting in a range of contrasting adaptation dynamics that can be analysed.

*Z. tritici* has developed various extents of resistance to fungicides with four of the five authorised modes of action in France: benzimidazoles (*e.g.* thiophanate-methyl, acting on microtubules), sterol-14α-demethylase inhibitors (DMIs; *e.g.* cyproconazole, epoxiconazole, metconazole, propiconazole, prothioconazole, tebuconazole), respiration complex III or cytochrome *b* inhibitors (QoIs; *e.g.*

azoxystrobin, pyraclostrobin, trifloxystrobin) and respiration complex II or succinate dehydrogenase inhibitors (carboxamides or SDHIs; *e.g.* benzovindiflupyr, bixafen, boscalid, fluopyram, fluxapyroxad, isopyrazam). The last group of fungicides, for which resistance has not yet developed, is multisite fungicides (*e.g.* chlorothalonil, folpet and mancozeb), which are known to be less prone to resistance development than molecules acting at single sites[11].

Resistance was selected and became generalized in the late 1980s for anti-microtubule agents (benzimidazole-resistant phenotype: BenR) and in the early 2000s for QoIs (strobilurin-resistant phenotype: StrR), resulting in field resistance and the progressive abandonment of these modes of action for treating STB in cereal crops in Western Europe. Resistance to SDHIs (carboxamide-resistant phenotypes: CarR) is just emerging in Europe and its frequency remains at or close to zero in most countries, including France. The BenR, StrR and CarR phenotypes are associated with unique point mutations of the genes encoding their targets (*i.e.* tub2 E198A[12]; cytb G143A[13]; many single changes in SdhB or SdhC), with a large effect on phenotype[14,15]. They are associated with qualitative resistance (a disruptive shift or single-step resistance), resulting in a bimodal distribution of phenotypes (individuals either sensitive or resistant), with the resistant phenotype rapidly invading the population[16]. By contrast, DMIs have been used intensively for STB control since the late 1970s; the first resistant strains were selected in the 1980s, and field efficacy has since gradually declined, but this decline has differed between molecules and between sites[17,18]. A continuum of decreased sensitivity phenotypes (triazole-resistant phenotypes: TriR) has arisen through the progressive accumulation of mutations in the *cyp51* target gene, with an overexpression of *cyp51* due to insertions in its promoter region, with or without enhanced efflux correlated with three possible insertions in the promoter region of the transporter MFS1[19–23]. This last mechanism induces a multidrug-resistant (MDR) pattern, with cross resistance between DMIs, QoIs and SDHIs[19]. The variable assortment of these independent mutations, possibly favoured by sexual reproduction, which occurs annually in this pathogen, and their selection after three decades of continuous DMI use, has led to highly diverse phenotypes in the field. This diversity accounts for the observed quantitative (progressive or slow-shifting), resistance with a multimodal distribution of phenotypes, succeeding and replacing each other, generally with an increase in resistance factors over time. More detailed information about resistance to the fungicides used to treat STB is provided in Supplementary Information 1.

Several phenotypic or molecular methods for detecting and quantifying fungicide resistance in *Z. tritici* have been reported and can be used to investigate resistance dynamics. Several studies have described the variation over space and/or time of the mean $EC_{50}$ (half maximal effective concentration: dose at which 50% of the maximal growth inhibition is observed) and its derivatives ("resistance intensity-based approaches"[16,18,24–28]), whereas other studies have described variations in the prevalence of several mutations or phenotypes ("resistance frequency-based approaches"[17,18,24,27,29–33]). Resistance intensity-based approaches characterize resistance phenotypes clearly, but require strain isolation, which is labour-intensive and time-consuming. These approaches are therefore subject to limitations due to the assessment of relatively small numbers of isolates sampled from the population, whereas other tools (*e.g.* molecular-based quantitative tools or microbiological tests using spore bulks) can quantify resistance frequency without the need for strain isolation, resulting in a more representative sample. Moreover, many of these studies have reported snapshot analyses of resistance status or changes in resistance over a short period[17,24,26,30,33] (~1-3 years) or in a limited territory[25,27–29,32,33] (~2-100 locations), using about 10-50 samples per date or location. Thus, in the studies published to date, the principal limitations to the description of resistance dynamics have been sample size and spatiotemporal scale, whereas the sample selected should be as exhaustive as possible, to

make it possible to obtain quantitative descriptions of resistance evolution.

Mathematical modelling can also be used to describe and to predict resistance, as it allows to compare the relative efficacy of different resistance management strategies. Most of the models reported to date describe the selection phase once the resistance has already emerged[34], but provide theoretical predictions of its evolution, and only one of the published models took field data into account[35]. Far fewer studies have focused on resistance emergence[5,36,37], but the first detection of resistance is now predicted reasonably well. Finally, these models focused on the temporal dimension and rarely take spatial heterogeneity into account[38–41], even though such heterogeneity of resistance is frequently observed in *Z. tritici* populations, especially at the scale of regional/national surveys[17,18,26,28,31,32,42]. This may result from the regional heterogeneity in crop environment but also in pesticide use. There is, therefore, a need for comprehensive biological datasets over appropriate and heterogeneous spatiotemporal scales and for models specifically designed for the quantitative description and prediction of resistance evolution in heterogeneous environments.

We retrospectively investigated the evolution of fungicide resistance in French populations of *Z. tritici*. We used a "frequency-based approach" comparing resistance dynamics between phenotypes for various fungicides corresponding to the different modes of action used for STB control, over wide temporal and regional spatial scales. We used the dataset provided by the "Performance" network, which has operated in France since 2004 providing the annual resistance status in the populations of *Z. tritici* sampled on winter wheat. A detailed statistical analysis was performed to study how phenotypes resistant to benzimidazoles, QoIs or DMIs dispersed spatially, the speed of such dispersion and differences between regions.

## 2 MATERIALS AND METHODS
### 2.1 POPULATION SAMPLING

#### 2.1.1 PERFORMANCE NETWORK DATASET
Most of the *Z. tritici* populations used for the analysis were provided by the "Performance" network. This trial network includes per year 37-70 very diverse partners, including technical institutes, advisers, merchants, farm co-operatives and chemical companies, and is run by the ARVALIS-Institut du Végétal (technical institute) and INRA BIOGER (for the resistance analyses). According to a common protocol applied annually between 2004 and 2017, this network recorded the efficacy and yield associated with 4-10 sets of fungicide spraying conditions, which were compared with unsprayed plots, in winter wheat. Trials consisted in a randomized block design with 3-4 replicates and a minimum of 20 m² for elementary plots. Then, the frequency of resistant phenotypes (see Section 2.2) was determined by analysing infected leaves collected from the plots. Administrative information (*e.g.* supplier, site and sampling date) and agronomic details (*e.g.* cultivar, leaf layer sampled) were also recorded. The network conducted 1029 trials in total between 2004 and 2017, throughout France. Sampling matched the pattern of winter wheat cultivation (Figure 1).
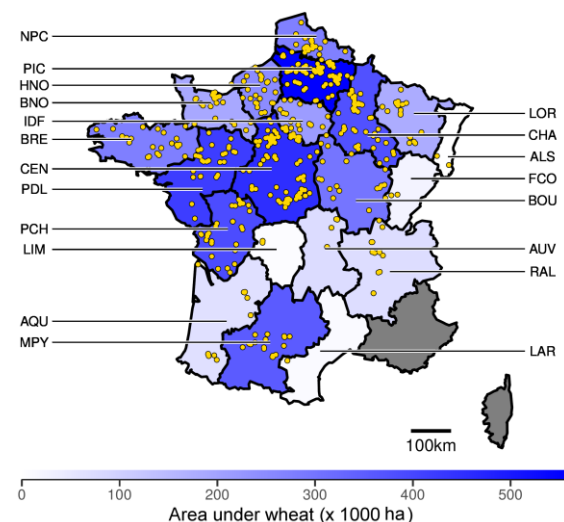


**Figure 1.** Distribution of *Performance* network trials between 2004 and 2017 in mainland France.

Dots represent trials and lines the regional structure before the reorganisation of French regions in 2015. Acronyms denote regions: ALS, Alsace; AUV, Auvergne; AQU, Aquitaine; BNO, Basse-Normandie; BOU, Bourgogne; BRE, Bretagne; CEN, Centre, CHA, Champagne-Ardennes; FCO, Franche-Comté; HNO,

Haute-Normandie; IDF, Ile-de-France; LAR, Languedoc-Roussillon; LIM, Limousin; LOR, Lorraine; MPY, Midi-Pyrénées; NPC, Nord-Pas-de-Calais; PCH, Poitou-Charentes; PDL, Pays de la Loire; PIC, Picardie; RAL, Rhône-Alpes. The colour gradient indicates the intensity of wheat cultivation in each region (agreste.agriculture.gouv.fr). The correlation between sampling effort and wheat production is strong (coefficient of 0.85). Regions are coloured in grey if no monitoring was performed.

We used resistance data of control plots only for this study. Indeed, as these plots were not treated with fungicides, their pathogen populations (*i.e.* the frequencies of the different resistances) can be considered representative of those in the surrounding environment. From 2006 to 2011, control plots were sampled twice: the first sample (T0; *n*=332 trials) was collected just before the first spray (at about stage Z32 or stem elongation phase, in mid-April to mid-May) and the second (TNT; *n*=719 trials) 20-30 days after the last spray (at about stage Z39-Z55 or booting and heading stages, in mid-May to mid-June). From 2004 to 2005 and from 2012 to 2017, only the later of these two samples was collected.

### 2.1.2 ADDITIONAL DATASETS
Some resistant phenotypes arose before 2004. We therefore retrieved, for this analysis, data from previous INRA monitoring programs and from the AFPP network (network of the French Association for Plant Protection). Additional information on resistance frequency was then considered for 1980, 1986, 1991, 1994, 1997 and 1999. For these years, mean resistance frequency was calculated from the observation of 10-20 plots annually, distributed throughout the area under wheat. These data were used to plot changes in resistance status over time, together with the Performance dataset, but were not considered in statistical models (see section 2.4).

## 2.2 RESISTANCE CHARACTERISATION
### 2.2.1 FUNGICIDES, MEDIA AND CHEMICALS
Fungicides were used as technical-grade compounds at one or two discriminating concentrations, and were kindly provided by their manufacturers: boscalid (1 ppm), carbendazim (5 ppm) and epoxiconazole (0.2 ppm) from BASF Agro (Germany); bixafen (0.5 ppm), prochloraz (0.005 ppm) and tebuconazole (0.05 ppm) from Bayer CropScience (Germany); azoxystrobin (0.5 ppm) and pyrifenox (0.01 and 0.15 ppm) from Syngenta Agro (Switzerland); triflumizole (0.032 ppm and 0.4 ppm) from Nippon Soda (Japan); terbinafine (0.01 ppm) from Sandoz (Switzerland). Tolnaftate (0.25 ppm) was purchased from Sigma Aldrich, St Louis (USA). These fungicides, at the doses indicated, formed a set of discriminatory doses, which were used to test the population. These doses were chosen on the basis of the dose-response curves for individual strains, in which the resistance-associated mutations of target genes were characterised. They were validated by showing that only strains bearing the resistant allele could grow in their presence. Inhibitors of sterol biosynthesis were selected so as to reveal the negative or positive cross-resistance specific to some genotypes.

Compounds were dissolved in 80% ethanol and added to molten medium after autoclaving. The concentration of ethanol in the medium, including that used for the controls without fungicides, was 0.5 mL $L^{-1}$. Penicillin (5 ppm) and streptomycin (5 ppm), both purchased from Sigma Aldrich (St Louis, USA), were added to the medium for all conditions tested, to prevent contamination with bacteria present on infected leaves.

The PG nutrient medium contained 10 g $L^{-1}$ glucose, 2 g $L^{-1}$ $K_2HPO_4$, 2 g $L^{-1}$ $KH_2PO_4$ and 12.5 g $L^{-1}$ agar (pH = 6.3). The medium, to which the antibiotics and fungicides were added at their discriminatory doses, was dispensed into 5.5 cm diameter Petri dishes.

### 2.2.2 MEASUREMENT OF RESISTANCE FREQUENCY
A "sample" was defined as a population constituted from pycnidiospores produced by 30-40 infected leaves, corresponding to the 3-4 repetitions of the control and kept dried at 4°C until use, one to three months after its collection. Leaves were cut into 2-3 cm pieces with scissors, soaked in 10 ml sterile water for ten minutes and then shaken vigorously. The leaf fragments were then removed. The concentration of pycnidiospores in each bulk

sample was estimated with an haematocytometer and adjusted to $5 \times 10^6$ spores ml$^{-1}$. For each population, 300 µl of the bulk spore suspension was spread over a set of Petri dishes, each dish containing a distinct discriminatory dose of fungicide, previously optimized on characterized pure strains to allow the growth of a given resistant genotype, or group of resistant genotypes (see below and Supplementary Information 2). Petri dishes were then incubated in the dark at 17°C. After 48 h of incubation, the dishes were observed under a light microscope (magnification x200). For each discriminatory concentration, sensitive pycnidiospores did not germinate or produced only short germ tubes, whereas resistant pycnidiospores produced long germ tubes (>40% control). Taking into account the percentage of the spores germinating in the control, we estimated the proportion of spores resistant to a given fungicide in the bulk with manual counts, based on ~100 spores in each Petri dish. We calculated the proportions of several phenotypes in the spore mixture. This protocol was used in every experiment during 2004-2017, which ensures the standardization and uniformity of the data. A full description of these phenotypes and associated genotypes is available from Leroux & Walker [19], but, in short, this test was found to be able to distinguish the following phenotypes, or groups of phenotypes:

- BenR phenotype (specific resistance to antimicrotubule agents): growth in 5 ppm carbendazim
- StrR phenotype (specific resistance to QoIs): growth in 0.5 ppm azoxystrobin
- CarR phenotype (specific resistance to SDHIs): growth in 1 ppm boscalid and 0.5 ppm bixafen
- TriR phenotypes (specific resistance to DMIs): growth in 0.01 ppm pyrifenox
  • TriLR phenotypes (low resistance to DMIs): no growth in 0.4 ppm triflumizole
    ▪ TriR1 and TriR3: no growth in 0.032 ppm triflumizole
    ▪ TriR5: no growth in 0.05 ppm tebuconazole

▪ TriR2 and TriR4: growth in 0.032 ppm triflumizole and 0.05 ppm tebuconazole
  • TriMR phenotypes (moderate resistance to DMIs): growth in TriLR conditions and in the presence of 0.4 ppm triflumizole
    ▪ TriR6: growth in 0.005 ppm prochloraz
    ▪ TriR7 and TriR8 (TriR7-TriR8): no growth in 0.005 ppm prochloraz
  • TriHR phenotypes (high resistance to some DMIs): growth in TriR6 conditions and in 0.15 ppm pyrifenox and 0.2 ppm epoxiconazole; no growth in 0.25 ppm tolnaftate and 0.01 ppm terbinafine
  • MDR phenotypes (resistance to DMIs; weak resistance to QoIs and SDHIs): growth in all TriHR conditions; growth in 0.25 ppm tolnaftate and 0.01 ppm terbinafine.

As correlations are established between each phenotype (defined as the ability to grow under a set of discriminatory concentrations) and a genotype or group of genotypes (Supplementary Information 2) and yearly updated with new genotypes, these phenotype groups can be used as proxies, to follow the evolution of specific mutations of target genes associated with resistance. Indeed, some mutations, or combinations of mutations, were specific to our phenotype groups.

### 2.2.3 STRAIN ISOLATION AND GENOTYPING
Each year, 50-100 strains were isolated from randomly selected populations (at about 20 sites). Infected leaves were placed in a damp chamber for 24 h to promote cirrhus exudation. Cirrhi were collected with a sterile needle and subcultured on malt-yeast agar medium (MYA: 20 g l$^{-1}$ malt extract, 5 g l$^{-1}$ yeast extract and 12.5 g l$^{-1}$ agar; pH = 5.9) three times, until purity was achieved. Isolated colonies with long germ tubes were also collected from media supplemented with the discriminatory concentrations described

above, with a sterile needle, under a stereomicroscope. Colonies were then purified as previously described. Pure strains were stored as spore suspensions in 20% glycerol at -80°C.

DNA was extracted and *cyp51* sequences were established as described by Leroux & Walker[19], to update the list of genotypes presented in Supplementary Information 2. We analysed the phenotype of pure strains of established genotype, as described above, checking against categories listed. As at least three genotypes (type I, II or III, depending on the length of the insertion in the *MFS1* promoter) may determine MDR, we established these genotypes systematically, for all strains phenotyped as MDR, by PCR with the primers described by Omrane *et al.*[22]. *sdhB, sdhC* and *sdhD* sequences were established after Dooley *et al.*[43].

## 2.3 ESTIMATION OF FUNGICIDE USE

Information about fungicide use collected via internal panels was provided by Bayer CropScience. Fungicide use was estimated from field surveys carried out on representative farms. The internal panels compiled, for each compound used for STB control, the amounts used in the region (in kg) and the corresponding deployed surfaces (in hectares). For a given fungicide, deployed hectares correspond to the number of sprays multiplied by the treated area, without regard to the dosage. For instance, 200 deployed hectares can mean either two applications of a single active ingredient over 100 ha, or one application of two different fungicides from the same mode of action at once over the same 100 ha. Panel data were available for all regions of France, from 1990 onwards. As an indicator of fungicide use, we calculated the national deployed hectares per mode of action (sum over all regions of the deployed surfaces for all the active ingredients sharing the same mode of action, used for STB control) divided by the area under wheat in France (from the AGRESTE database: agreste.agriculture.gouv.fr). This indicator, denoted $HA_{\frac{D}{C}}$ (deployed hectares divided by cultivated hectares), represents the mean number of sprays of a mode of action in France for a given year. As the use of SDHIs is recommended to one spray per year in France, $HA_{\frac{D}{C}}$ for SDHIs represents the proportion of surfaces sprayed with this class of fungicides.

**Table 1.** Data used in the statistical analysis, extracted from the *Performance* database.

| Phenotype | | First year observed | Number of years observed | Number of regions monitored | Number of regions monitored yearly | Number of observations |
|---|---|---|---|---|---|---|
| BenR | | 2007 | 11 | 16 | 6 | 772 |
| StrR | | 2004 | 9 | 16 | 9 | 860 |
| TriLR | Total | 2005 | 12 | 16 | 7 | 963 |
| | TriR2-TriR4 | 2006 | 10 | 16 | 9 | 860 |
| | TriR5 | 2006 | 10 | 16 | 9 | 861 |
| TriMR | Total | 2005 | 13 | 16 | 6 | 985 |
| | TriR6 | 2006 | 12 | 16 | 6 | 912 |
| | TriR7-TriR8 | 2006 | 12 | 16 | 6 | 853 |
| TriHR | | 2010 | 8 | 14 | 6 | 360 |
| MDR | | 2013 | 5 | 6 | 4 | 116 |
| **Total** | | | | | | **7542** |

## 2.4 STATISTICAL METHODS FOR ASSESSING RESISTANCE DYNAMICS
### 2.4.1 GENERAL FEATURES OF STATISTICAL MODELS

The frequency of each resistant phenotype in *Z. tritici* populations was considered as the dependent variable in our models. We excluded only the TriR1-TriR3 and CarR phenotypes, for which we had too few non-

zero observations for modelling (*n*=23 and *n*=4 observations, respectively) in our dataset relative to other phenotypes (n=755 observations in average, see Table 1). We used a logistic regression framework where the observations followed a binomial distribution of probability *p* and where the logit of *p* ($logit(p) = \ln(\frac{p}{1-p})$) is a linear combination of explanatory factors. Three models were studied here: a SPATIAL MODEL to determine the spatial structure of each resistant phenotype; an ANOVA MODEL to evaluate the variability of frequencies among years and regions; and a DYNAMIC MODEL to model the dynamics of resistant proportions. The SPATIAL MODEL was considered in a frequentist framework, whereas the ANOVA and DYNAMIC MODELS were analysed in a Bayesian framework.

We considered three main factors in these models: year, region and sampling date. For sampling date, TNT (early summer samplings) was used as the reference (*i.e.* this parameter was set to 0), because TNT was observed every year in the dataset. We therefore actually estimated the difference between TNT and T0 (mid-spring samplings) effects within a year.

In this study we focused on periods and locations for which resistance was evolving (*i.e.* after emergence and before fixation). Thus, we selected appropriate years and regions among the dataset. For each phenotype, we defined a time range gathering years for which at least a quarter of the regions were monitored with frequencies differing from 0% and 100% (see time ranges in Table 1). In ALS, FCO, LAR and LIM regions, only one trial per year was conducted over three years or less, unlike in other regions where three trials were conducted over at least five years. For the ANOVA and DYNAMIC MODELS, these four regions were removed from the dataset for all the phenotypes. Then, for each phenotype, we selected regions whose frequency data differed from 0% and 100% during at least a quarter of the years of the previously defined time range (see the number of remaining regions in Table 1). Finally, this selection only removed a bit less than 5% of the observed data that were different from 0% and 100%.

Due to the *logit* link function involved in these models, values of 0 and 100 were overweighted, making parameters difficult to estimate for several phenotypes (*e.g.* when resistance was emerging or when resistance was completely generalised). We therefore added 0 and 100 inflation parameters[44,45] to the last two models. These two inflation parameters are linked to the proportions of 0 and 100 values observed in the dataset.

The models described below were run for each phenotype, with over-dispersion parameters in the binomial distribution for the ANOVA and DYNAMIC MODELS. Statistical analyses were performed with *R* software[46] and the *rjags* package[47] for Bayesian statistics. Parameter estimates were considered significant at the 5% level (or, alternatively, the 2.5% or 0.1% level) if 0 lay outside the 95% (97.5% or 99.9%, respectively) confidence interval for the parameter. Qualitative factors were tested at the same significance levels (5%, 2.5% and 0.1%), using Wald tests[48]. The goodness-of-fit of models was assessed using the coefficient of determination ($R^2$) which varies between 0 and 1. We used the following definition of the $R^2$: $\frac{SSM}{SST}$, where SSM corresponds to the explained sum of squares (squared differences between predictions and the average of observations), and SST corresponds to the total sum of squares (squared differences between observations and the average of observations). The closer its value is to 1, the better the goodness-of-fit will be.

### 2.4.2 MODELLING THE SPATIAL STRUCTURE OF RESISTANCE
This model was designed to visualize the spatial clustering of resistances over time on individual maps. We used a geostatistics method to interpolate from sparse sample data by kriging to produce maps along a regular grid[49]. This method principle is that « data that are close together are usually more correlated than those that are far apart »[50]. The spatial dependency of variables is modeled by a variogram (a function only dependent on distance). We estimated variogram parameters using a Matérn space covariance matrix[51]

modelling the covariance among observations in a generalized linear mixed model[52] taking year and sampling date into account as qualitative factors. Using the estimated variogram, we obtained by kriging an unbiased linear prediction of minimum variance[50] of resistance frequencies over the monitored area for a given year. We then used the SpODT method (spatial oblique decision tree) to estimate lines that delimit areas as different as possible[53]. Based on a non-parametric method of classification, this algorithm partitions the area by a line that gives the two spatial classes which maximize the interclass variance. This analysis involved the use of two packages: *spaMM*[54] for spatial interpolation, and *SPODT*[53] for spatial partitioning, and is referred to hereafter as the "SPATIAL MODEL".

## 2.4.3 MODELLING VARIATIONS OF RESISTANCE FREQUENCY BETWEEN YEARS AND REGIONS

This second model was designed to identify specific years or regions deviating significantly from an estimated national mean resistance frequency. This model ranked years and regions according to their observed resistance frequencies. Year and region were considered as qualitative factors. The interaction between year and region, and the impact of sampling date in control plots (T0 *vs.* TNT) were also included in this model. The statistical form of the model is given in Supplementary Information 3.1 and this model is referred to hereafter as the "ANOVA MODEL". For each phenotype, the estimated model parameters permit to compute adjusted mean i.e. to compensate for data imbalances. Thus, the interannual and national mean frequency is given by $(1-\pi_0-\pi_{100})*(\text{logit}^{-1}(\mu) + \pi_{100}$, where $\text{logit}^{-1}(x)= \exp(x)/(1+\exp(x))$ and $\mu$ is the intercept of the model. The mean frequency for T0 (mid-spring samplings) is given by $(1-\pi_0-\pi_{100})*\text{logit}^{-1}(\mu-\alpha_{\text{TNT-T0}})) + \pi_{100}$, where $\alpha_{\text{TNT-T0}}$ is the effect of the sampling date. The adjusted frequency for the year $j$ is given by $(1-\pi_0-\pi_{100})*(\text{logit}^{-1}(\mu+\alpha_j) + \pi_{100}$ where $\alpha_j$ is the effect of the year $j$. The adjusted frequency for the region $k$ is given by $(1-\pi_0-\pi_{100})*(\text{logit}^{-1}(\mu+\beta_k) + \pi_{100}$ where $\beta_k$ is the effect of the region $k$. More generally, the frequency estimated for a year $j$

and a region $k$ is $(1-\pi_0-\pi_{100})*(\text{logit}^{-1}(\mu+\alpha_j+\beta_k) + \pi_{100}$.

## 2.4.4 MODELLING THE RATE OF INCREASE IN RESISTANCE

The aim of this third model was to estimate and compare the rates of evolution of the different phenotypes in France and between regions. As above, the model included year, region and sampling date effects, but year was treated here as a quantitative variable. The model was therefore dynamic because the years were ordered. This model provided estimates of growth rates of resistance frequency (hereafter named "growth rate") at national and regional scales. The exponential of these growth rates could be interpreted as the apparent relative fitness of phenotypes or, in other words, how much faster the resistant phenotype evolved than the rest of the population[55]. As we consider a relative growth, estimates will be close to 1 if the frequency of the phenotype remains stable in the population, above 1 if the frequency increases over time and below 1 otherwise. The statistical form of the model is given in Supplementary Information 3.2. This model is hereafter referred to as the "DYNAMIC MODEL".

# 3 RESULTS

## 3.1 COMPARISON BETWEEN SAMPLING DATES

For each phenotype, the ANOVA and DYNAMIC MODELS gave similar estimates of the "TNT-T0" parameter. This parameter informs on the intra-annual evolution of resistance frequency in control plots by estimating a constant difference between mid-spring and early summer samples (Table 2 and Supplementary Information 4 and 5). Hereafter, we therefore provided the results for the ANOVA MODEL only. Estimates were significantly positive for the StrR, TriR6 and TriMR phenotypes, with increase in frequencies between TNT and T0 of 4.7% ($P < 0.001$), 8.9% ($P < 0.001$) and 3.5% ($P < 0.025$), respectively (Table 2). By contrast, estimates were significantly negative for the TriR2-TriR4 and TriLR phenotypes, with decrease in frequencies between TNT and T0 of

-1.87% (*P* < 0.025) and -2.2% (*P* < 0.025), respectively (Table 2).

Furthermore, a strong positive correlation (Pearson's correlation coefficient of 0.82, *P* = 0.004) was found between estimated "TNT-T0" parameter and the national growth rate, for all phenotypes. The frequency of resistance between T0 and TNT (intra-annual variation) thus followed the same pattern as general inter-annual variation. The correlation coefficient reached 0.90 (Pearson's correlation coefficient, P = 0.006) if only the phenotypes displaying significant inter-annual evolution were considered (Supplementary Information 5).

We focus below on TNT observations, unless otherwise indicated.

**Table 2.** Frequencies and frequencies differences estimated from the ANOVA model for year, region and sampling date effects.

| Factor | | Phenotype | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BenR | StrR | TriLR | TriR2-TriR4 | TriR5 | TriMR | TriR6 | TriR7-TriR8 | TriHR | MDR |
| | Inter-annual national mean frequency % | 89.90*** | 89.63* | 11.01*** | 7.64*** | 4.47*** | 78.31*** | 56.07 | 17.50*** | 7.08*** | 4.69*** |
| Year | *Tests (p.values)* | *0.894* | *0\*\*\** | *0\*\*\** | *0.065 ·* | *0\*\*\** | *0\*\*\** | *0\*\*\** | *0\*\*\** | *0.034\** | *0.972* |
| | 2004 | | -42.87** | | | | | | | | |
| | 2005 | | -25.27* | 27.95*** | | | -16.53** | | | | |
| | 2006 | | -8.99 | 13.55* | 2.38* | 2.25 | -4.43 | -3.4 | -0.95 | | |
| | 2007 | -1.85 | -5.17 | 9.66· | 2.04· | 4.04* | -0.84 | -3.4 | 2.31 | | |
| | 2008 | -1.27 | -5.06 | 9.66· | 2.38* | 2.88* | -0.5 | -3.4 | 1.28 | | |
| | 2009 | 1.15 | -2.84 | 3 | -0.2 | 2.38· | 6.2* | 10.6 | -4.35 | | |
| | 2010 | 0.26 | 4.84 | 1.32 | -1.26 | 1.05 | 5.94* | 10.6 | -4.46 | -5.19** | |
| | 2011 | 0 | 6.52· | -0.57 | -1.08 | -0.16 | 7.21* | 11.76 | -2.48 | -2.87 | |
| | 2012 | 1.9 | 8.3*** | -0.38 | -0.27 | -1.06 | 2.7 | 7.58 | -8.12** | -2.2 | |
| | 2013 | 1.31 | | 1.86 | 1.31 | -0.4 | 0.66 | -12.57* | 8.23* | 0.31 | -0.69 |
| | 2014 | 0.26 | | -4.25 | -1.92 | -2.18· | 2.39 | 9.61 | -2.36 | 2.67 | -0.32 |
| | 2015 | -1.66 | | -8.19** | -1.81 | -3.13** | 5.54 | 8.4 | 5.15 | 0.84 | 0.99 |
| | 2016 | 0.26 | | -10.31*** | | | -10.41* | -3.17 | -6.32 | 9.35* | 0.43 |
| | 2017 | -0.8 | | | | | -7.54 | -32.74*** | 25.04*** | 6.46· | -0.2 |
| Region | *Tests (p.values)* | *0\*\*\** | *0.224* | *0.004\*\** | *1* | *0.018\** | *0.99* | *0.01\** | *0\*\*\** | *1* | *1* |
| | NPC | 4.06* | 5.22* | -3.93* | -0.4 | -1.43 | 2.7 | 11.38* | -7.25* | 2.36 | -0.08 |
| | PIC | 3.95** | 2.89 | -4.56* | -1.14 | -1.93* | 2.55 | 8.6· | -6.12· | 1.83 | 0 |
| | HNO | 4.23· | 4.3* | -2.59 | -0.4 | 0.08 | 2.39 | 9.41· | -5.21 | 0.06 | |
| | BNO | 1.46 | 1.55 | 0.8 | 0.63 | -0.28 | -2.41 | 3.98 | -7.43* | 2.75 | |
| | LOR | 2.67 | -0.15 | 1.53 | 0 | -0.65 | -0.5 | 7.37 | -5.52 | -1.09 | |
| | CHA | 1.97 | 1.3 | -0.47 | -0.27 | -0.72 | 0.82 | 6.33 | -5.42· | 0.97 | -0.12 |
| | IDF | 2.12 | 2.68 | -0.93 | -0.2 | 0.13 | 1.14 | 11.19* | -5.52 | -1.5 | -0.2 |
| | BRE | -7.25** | 1.43 | -2.36 | -0.14 | -1.19 | 1.14 | -5.23 | 6.49 | 0.77 | |
| | CEN | 0.26 | 2.53 | 3.73* | 0.14 | 1.85· | -1.19 | -0.68 | -2.11 | -1.89 | 0.04 |
| | PDL | -4.89· | -11.57* | 3 | 0.14 | 3.2* | -2.95 | -8.45· | 4.66 | -0.3 | 0.38 |
| | BOU | 0.42 | -1.76 | 1.75 | 0.35 | 0.66 | -0.33 | -0.9 | -1.86 | | |
| | PCH | -1.85 | -4.42 | 6.79** | -0.27 | 3.61** | -3.87 | -10.29 | 10.23* | -0.76 | |
| | AUV | 0.34 | -0.68 | 2.42 | 0.63 | 0.76 | -2.95 | -1.81 | -2.85 | 0.97 | |
| | RAL | -3.09 | -0.76 | 1.43 | 0.28 | -0.12 | -0.5 | 3.98 | -0.95 | -1.79 | |

The table gives the inter-annual national mean frequency and the difference between the adjusted frequency and the mean frequency for each level of each factor. Frequencies are expressed in percentage. Estimates of interactions between region and year are not shown because the factor was not significant for all phenotypes. Empty cells indicate that no data were available for estimation. Factors tests *P*-values are indicated in bold and italic. *P*-value thresholds: "·" (*P* < 0.1), "*" (*P* < 0.05), "**" (*P* < 0.025), "***" (*P* < 0.001).

## 3.2 RESISTANCE TO ANTIMICROTUBULE AGENTS (BENZIMIDAZOLES)

Benzimidazoles were introduced into France in the mid-1970s to control STB (Supplementary Information 1). They have marginal use on wheat with an $HA_{\frac{D}{c}}$ index of 0.0081 on average over the last five years (residual use against *Fusarium* head blight). Over the last decade, the frequency of BenR in *Z. tritici* populations has remained very high and stable (Figure 2), as already observed in the 1990s[56]. Inter-annual national frequency has been estimated at 90% (Table 2). The stabilisation of this resistance is confirmed by the lack of a significant effect of the year factor (Table 2) and by an estimated increase in frequency not significantly different from 1 at national scale (Figure 3).
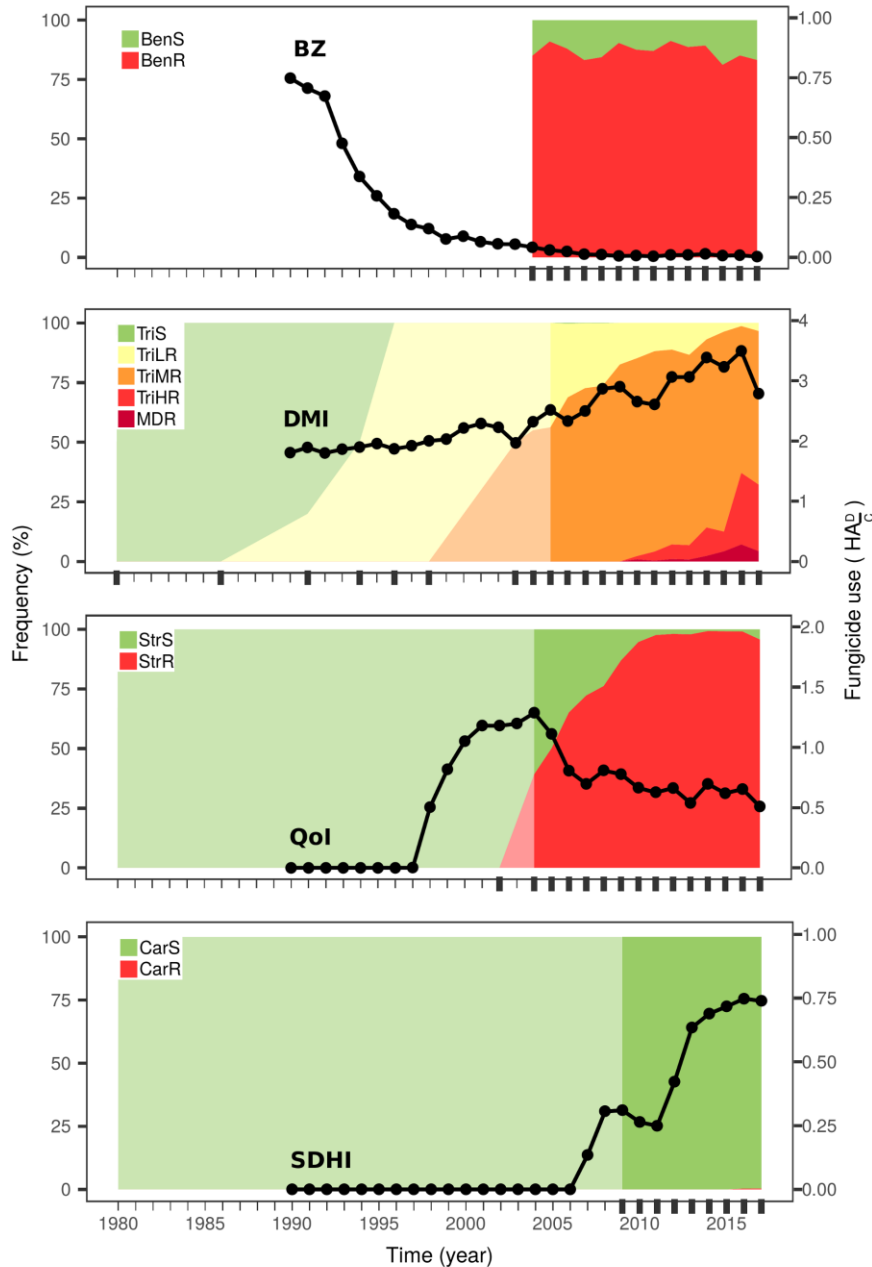


**Figure 2.** Changes in fungicide use and resistance frequency in *Z. tritici* populations in France.
Thick ticks along the *x*-axis indicate years for which resistance frequency was assessed by monitoring. Lighter ticks indicate that information was obtained or extrapolated from sources other than the *Performance* database. BZ: benzimidazoles (antimicrotubule agents); DMI: sterol demethylation inhibitors; QoI: inhibitors or respiration complex III; SDHI: inhibitors of respiration complex II. Phenotypes as described in section 2.2.

A regional effect was detected, with differences between the western and northern regions ($P < 0.001$; Table 2; Supplementary Information 6.1). Indeed, the estimated frequency of resistance was significantly lower than the inter-annual national mean in the AQU and BRE regions (-10.75% ($P < 0.025$) and -7.25% ($P < 0.025$), respectively). Resistance frequency was significantly higher than the inter-annual national mean in the NPC and PIC regions (+4.1% ($P < 0.05$) and +3.9% ($P < 0.025$), respectively). The rate of increase in the frequency of BenR was similar to the national trend for all regions, with the exception of PDL, which displayed a greater increase in frequency than the other regions (estimate = 0.07; $P < 0.05$), and CEN, in which the increase in the frequency of BenR was significantly lower and negative (estimate = -0.07; $P < 0.05$) (Figure 3 and Supplementary Information 5).
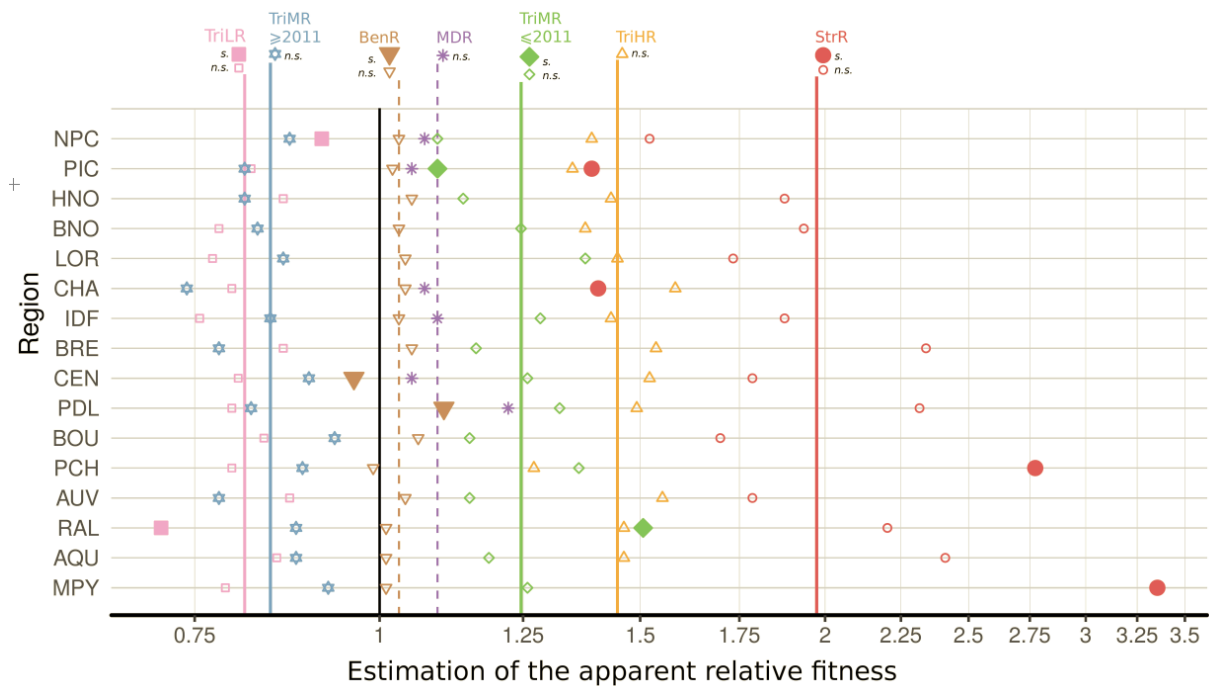


**Figure 3.** Apparent relative fitness estimates (increases in frequency of resistance) for different resistant phenotypes.
The apparent relative fitness is equal to the exponential of frequency growth rate from the DYNAMIC MODEL, and it represents how much faster the resistant phenotype evolved compared to the rest of the population (see Section 2.4.4). When the apparent relative fitness is close to 1, resistance frequency tends to be constant in the population. Vertical lines: apparent relative fitness estimates at the national scale for each phenotype; lines are continuous if parameters are significant ($P < 0.05$), dashed otherwise. Dots: estimates of the regional adjustment of the national apparent relative fitness; *s.* means significant ($P < 0.05$), *n.s.* means non-significant. Regions on the vertical axis are organized according the North-South gradient.

## 3.3 RESISTANCE TO QUINONE OUTSIDE INHIBITORS (QoIs)

QoI fungicides were first used to control STB in France in 1997 (Supplementary Information 1) and the first resistant strains were detected in 2002[56]. QoIs use is currently half as high as in the early 2000s. It decreased substantially between 2005 and 2007 because of their poor efficacy on resistant populations (Figure 2). The remaining use of fungicides with this mode of action is chiefly to control rusts and *Fusarium* head blight.

The national frequency of StrR increased sharply between 2002 and 2012, as shown by the estimates of the year parameters in the ANOVA MODEL (from -43% in 2004, to + 8.3% ($P < 0.001$) in 2012), with a frequency of 95% already attained in 2010 (Table 2). The DYNAMIC MODEL confirmed this trend, with a significant and positive growth rate of 0.68 ($P < 0.001$, Supplementary Information 5), indicating that the StrR population grew twice ($e^{0.68} \approx 2$) as fast as the rest of the population over the study period (Figure 3). Furthermore, StrR increased

in frequency significantly more rapidly than all the other phenotypes (at least $P < 0.05$, data not shown).

The ANOVA MODEL (Table 2) detected a spatial structure, with northern regions, such as NPC and HNO, having a significantly higher StrR frequency on average over time (+5.2% and +4.3%, respectively; $P < 0.05$), and south-western regions, such as AQU and PDL, having a lower StrR frequency on average over time (-13.2% ($P < 0.1$) and -11.6% ($P < 0.05$), respectively).

Based on the output of the DYNAMIC MODEL (Figure 3 and Supplementary Information 5), increases in frequency were significantly lower than the national rate for the northern PIC and CHA regions (estimates = -0.35 ($P < 0.001$) and -0.34 ($P < 0.05$), respectively), whereas the south-western regions MPY and PCH had significantly higher rates of frequency increase (estimates of 0.53 ($P < 0.025$) and 0.34 ($P < 0.05$), respectively). The coefficient of determination ($R^2$) for this model is much higher (0.86) than those of the other phenotypes (TriR6: 0.53, TriHR: 0.52, TriLR and TriR5: 0.51, MDR: 0.49, TriR7-TriR8: 0.48, TriMR≤2011: 0.45, BenR: 0.4, TriR2-TriR4: 0.33 and TriMR≥2011: 0.25; see Supplementary Information 5).

Resistance frequency followed a north-south gradient between 2004 and 2008, with clear delimitations (advancing front) highlighted by the SPATIAL MODEL (Figure 4 and Supplementary Information 6.2). The speed of the progression line (*i.e.* the distance between the fronts of two successive years) was 120-145 km/year (depending the timeframe used: 2004-2007 or 2004-2008).

## 3.4 RESISTANCE TO SUCCINATE DEHYDROGENASE INHIBITORS (SDHIs)

SDHIs were first used to control STB in France in 2007 (boscalid, a pyridine-carboxamide). The group of SDHIs available for application increased with the release of pyrazole-carboxamides (*e.g.* benzovindiflupyr, bixafen, fluxapyroxad, penthiopyrad) from 2011, and of the benzamides fluopyram in 2017 (Supplementary Information 1). The first strain displaying specific resistance (CarR; genotype SdhC-T79N) to be isolated in France was obtained in 2012 (source: FRAC). CarR strains were detected only four times and at low frequency (but not isolated) in our study: in a population sampled in PDL in 2016 (10% frequency) and then in three populations from CHA, PIC (5%) and NPC (2%) in 2017. SDHIs use was stable between 2008 and 2011, with 25% of the surfaces under wheat sprayed with at least one SDHI (Figure 2). It increased again to reach 75%, since 2012, *i.e.* at the time when pyrazole SDHIs, exhibiting higher intrinsic activity, were authorized in France.

Due to the rarity of CarR strains in our dataset, we did not run any models for this phenotype. Sequencing of pure strains (2013-2018) pinpointed the changes N225I in SDH-B, T25N, I29V, N33T, N34T, T79N, H152R in SDH-C, and T18N, A126D, D129G and K186R in SDH-D, compared to the IPO-323 reference sequence. The changes at codons T79 and H152R (SDH-C) and D129 (SDH-D) are associated with lower SDHI sensitivity[14,57,58].

## 3.5 RESISTANCE TO STEROL 14α-DEMETHYLATION INHIBITORS (DMIs)

DMIs include imidazoles (*e.g.* prochloraz), triazoles (*e.g.* cyproconazole, epoxiconazole, metconazole and propiconazole) and a triazolinethione (prothioconazole, which is metabolised to generate prothioconazole-desthio, a triazole). These compounds have been used to control STB on winter wheat in France since the early 1980s, with new molecules regularly released onto the market (Supplementary Information 1). DMIs are the most widely used fungicides in France compared to the other modes of action. We noticed a steady increase in the use of this mode of action, with the index $HA_{\frac{D}{c}}$ ranging from ~2 in the early 1990s to over 3 currently (Figure 2). This observation suggests an increase in DMIs doses and/or in the number of compounds mixed in spraying treatment.

### 3.5.1 TriLR

TriLR phenotypes include strains with weak specific resistance to DMIs: phenotypes TriR1

to TriR5. These strains were first detected in the late 1980s (Figure 2). As we had too few non-zero frequencies observed for TriR1 and TriR3, we focused our analysis on the TriLR group in general and on phenotypes TriR2, TriR4 (grouped into the TriR2-TriR4 group) and TriR5. The estimated inter-annual national frequency of TriLR according to the ANOVA MODEL was 11% (7.6% for TriR2-TriR4 and 4.5% for TriR5; Table 2). For TriLR, annual estimates decreased from +28% in 2005 ($P < 0.001$) to -10.3% in 2016 ($P < 0.001$). This trend was also valid for TriR5 ($P < 0.001$, for the year factor), but was less pronounced for TriR2-TriR4 ($P < 0.1$, for the year factor). The national rates of frequency increase from the DYNAMIC MODEL confirmed these trends, as they were significantly negative for TriLR, TriR2-TriR4 and TriR5 (estimates of -0.21, -0.1 and -0.14, respectively; $P < 0.001$; Figure 3 and Supplementary Information 5).

Spatial differentiation was significant for TriLR and TriR5 ($P < 0.05$, for the region factor in the ANOVA MODEL), but not for TriR2-TriR4 (Table 2). Similar estimates were obtained for the TriLR and TriR5 phenotypes, with differences between northern regions and central-western regions (Table 2 and Supplementary Information 6.3, 6.5). For example, the region parameters for TriLR were significantly negative for PIC and NPC (-4.6% and -3.9% respectively; $P < 0.05$). This phenotype was therefore less present in these regions than in other regions over the study period. By contrast, it was significantly more present in the PCH and CEN regions (+6.8% ($P < 0.025$) and +3.7% ($P < 0.05$), respectively).

For the TriR2-TriR4 and TriR5 phenotypes, none of the regional rates of increase in frequency differed significantly from the national trend (Supplementary Information 5). Nevertheless, the rate of increase in frequency of the TriLR phenotype was significantly less negative than the national rate in the northern region NPC (estimate = 0.12; $P < 0.05$), whereas significant counter-selection against this phenotype was observed in the southern region RAL (estimate = -0.13; $P < 0.05$). Overall, there was counter-selection against TriLR strains nationally during the timeframe of this

study, because all regional growth rates (the sum of the national growth rate and regional adjustments, Supplementary Information 5) were negative.

### 3.5.2 TriMR

TriMR strains display moderate resistance to azoles. They include the TriR6, TriR7 and TriR8 phenotypes, which were selected in the late 1990s (Figure 2). Between 2005 and 2017, the estimated inter-annual national frequency of TriMR was 78% (with 56% for TriR6 and 17.5% for TriR7-TriR8). TriMR, and TriR6 in particular, were the most frequent group of TriR strains between the mid-2000s and 2017 (Table 2).

The ANOVA MODEL provided increasing estimates of annual frequency for TriMR between 2005 (-16.5% 0.83 in 2005; $P < 0.025$) and 2011 (+7%; $P < 0.05$). These estimates then decreased to reach -10.4% in 2016 ($P < 0.05$). This dynamic led us to split the TriMR data into two subgroups (TriMR≤2011 and TriMR≥2011), to estimate growth rates more reliably over these two periods.

The DYNAMIC MODEL estimated a significant positive growth rate of 0.22 for the period before 2011 ($P < 0.001$), indicating that this population of strains grew 1.25 times ($e^{0.22} \approx 1.25$) faster than the rest of the population (Figure 3). The rate of increase in frequency was significantly negative after 2011 (estimate = -0.17; $P < 0.025$).

By contrast, no significant trends were observed for the TriR6 and TriR7-TriR8 phenotypes in the DYNAMIC MODEL (non-significant growth rates close to 0, Supplementary Information 5). However, the structure of the TriMR group varied over time. Indeed, the estimates of some year parameters were significant for TriR6, and either positive, when this phenotype was present at higher frequencies than the inter-annual national mean (as in 2009, 2010 and 2011; $P < 0.05$, about 67% on the frequency scale), or negative, when this phenotype was present at lower frequencies (as in 2013 and 2017; $P < 0.05$ and $P < 0.001$ respectively, 44% and 23% on the frequency scale). Conversely, the TriR7-TriR8 phenotypes were present at higher

frequencies than the inter-annual national mean in 2013 and 2017 ($P < 0.05$ and $P < 0.001$ respectively, 26% and 43% on the frequency scale), and lower frequencies in 2012 ($P < 0.025$, 9% on the frequency scale).

The SPATIAL MODEL revealed a strong geographical partitioning of TriMR subgroups, with TriR6 strains found mostly in north-eastern regions, and TriR7-TriR8 strains found mostly in south-western areas, particularly before 2014 (Figure 4 and Supplementary Information 6.7-8). Furthermore, estimates of TriR6 frequencies (ANOVA MODEL) were significantly greater in the northern regions NPC and IDF, than the inter-annual national mean frequency (+11.4% and +11.2% respectively; $P < 0.05$). By contrast, estimates for the TriR7-TriR8 phenotypes in the southern regions MPY, AQU and PCH were significantly higher than the inter-annual national mean frequency (+35.3% ($P < 0.025$), +16.2% ($P < 0.05$) and +10.2% ($P < 0.05$), respectively). Estimates for the northern regions BNO and NPC were significantly below the inter-annual national mean frequency (estimates of -7.4% ($P < 0.05$) and -7.25% ($P < 0.05$), respectively).
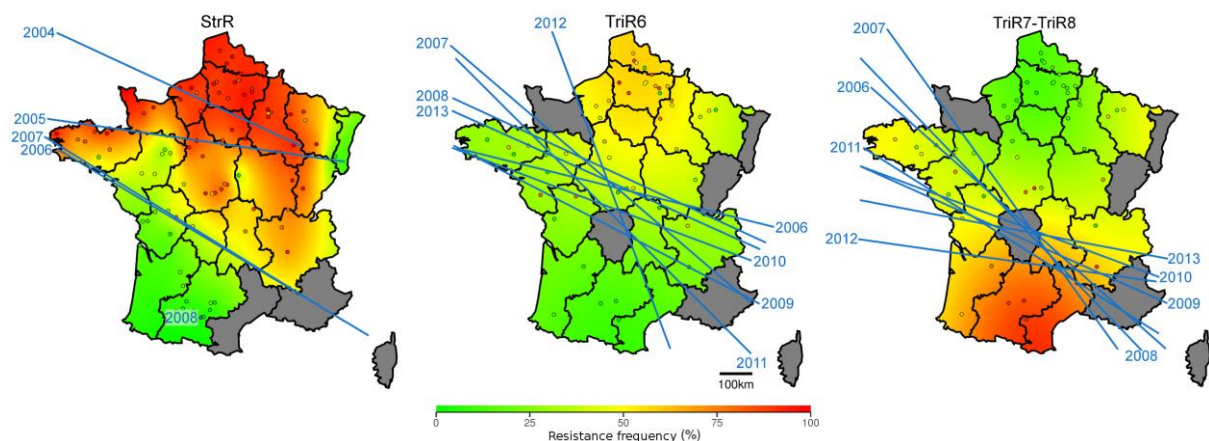


**Figure 4.** Spatial partitioning over time for the StrR, TriR6 and TriR7-TriR8 phenotypes in France. Background maps with colour gradient are obtained by kriging methods, from real observations (points). The colours within the observation points indicate the true frequency observed in trials. The lines are obtained by the SPODT method (spatial oblique decision tree[53]) and split space into two homogeneous blocks, one with lower frequencies than the other (or no resistance at all for the StrR phenotype). Left: background map and points representing the status of StrR resistance in 2006; colonization fronts from 2004 to 2007 are shown; for 2008, we show only the farthest trial from the 2007 front, because no more trials were found with frequencies close to 0% in this year. Middle: background map and points representing the status of TriR6 resistance in 2013; delimitation lines from 2006 to 2013 are shown. Right: background map and points representing the status of TriR7-TriR8 resistance in 2013; delimitation lines from 2006 to 2013 are shown.

Contrasting selection patterns were observed between regions with the DYNAMIC MODEL (Figure 3 and Supplementary Information 5) for the TriMR phenotypes, particularly for the TriR6 and TriR7-TriR8 subgroups. For example, TriR6 strains were selected, as shown by the positive rates of increase in their frequency, in RAL (estimate = 0.16; $P < 0.05$) and PCH (estimate = 0.13; $P < .05$), but counter-selected in MPY (estimate = -0.15; $P < 0.05$) and CHA (estimate = -0.13; $P < 0.025$). Similarly, TriR7-TriR8 phenotypes were selected in MPY (estimate = 0.22, $P < 0.001$) and BOU (estimate = 0.14; $P < 0.05$), but counter-selected in IDF (estimate = -0.16; $P < 0.05$).

### 3.5.3 TriHR
TriHR strains are highly resistant to some DMIs and are associated with the most complex *cyp51* genotypes (Supplementary Information 2). They were first detected in our dataset in 2008. Since 2010, their frequency has increased, and the estimated inter-annual national frequency of these strains was 7% (Table 2). According to the ANOVA MODEL, TriHR frequency increased from 1.9% in 2010 (7.1-5.2%, $P < 0.025$) to 16.4% in 2016 ($P < 0.05$). The DYNAMIC MODEL confirmed this national tendency, with a significant positive rate of increase in frequency of 0.37 ($P < 0.001$), indicating that the TriHR population grew

almost 1.5 times ($e^{0.37} \approx 1.5$) faster than the rest of the population (Figure 3).

No significant differences in TriHR frequency between regions were noted over the studied period (Table 2). Similarly, no regional adjustment parameter for the national rate of increase in frequency was significant (Figure 3). Maps from the SPATIAL MODEL are provided in Supplementary Information 6.9.
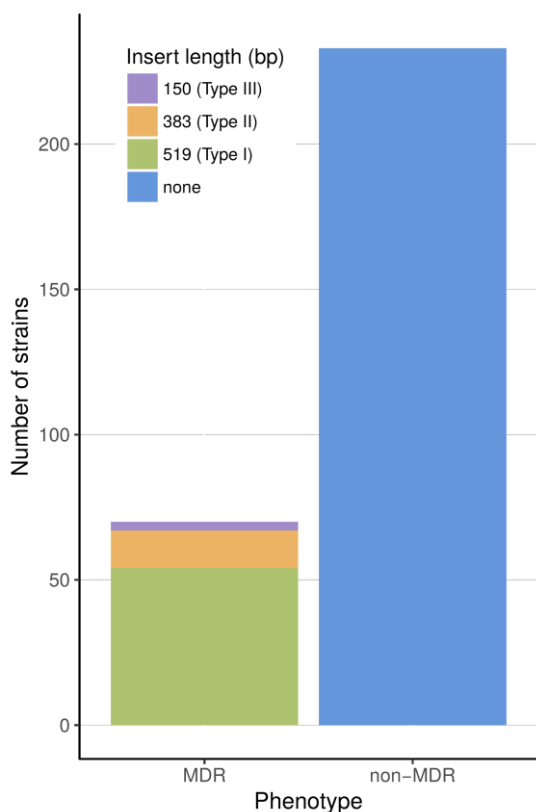


**Figure 5.** Distribution of mutations (insertion into the promoter of the MFS1 transporter gene) among MDR and non-MDR phenotypes in isolated *Z. tritici* strains (2008-2017; n=303).

### 3.6 MULTIDRUG RESISTANCE

In France, MDR phenotypes were first detected in 2008, at about the same time as TriHR strains emerged. MDR is based on an original mechanism of enhanced efflux, giving rise to cross-resistance between DMIs, QoIs and SDHIs. As this mechanism was selected in TriR and StrR backgrounds, resistance factors are high only for DMIs and QoIs, due to the combination of resistance mechanisms. The national frequency of MDR over the study period was estimated at 4.7% (Table 2). No significant year or region effects able to

account for the spatiotemporal distribution of this phenotype were identified (Table 2). MDR frequency tended to increase in France, but this trend was not significant (Figure 3 and Supplementary Information 5). Likewise, there was no significant regional effect on the national rate of increase in the frequency of MDR (Figure 3). Maps from the spatial model are provided in Supplementary Information 6.10.

The systematic genotyping of pure MDR strains collected since 2008 revealed that the type I mutation (519 bp insertion in the promoter region of *MFS1*) was the most frequent allele associated with MDR in French populations (frequency greater than 75% in the dataset; Figure 5).

## 4 DISCUSSION

We performed a retrospective study of changes in resistance frequency in *Z. tritici* populations collected from control plots in the *Performance* national field trial network. The populations studied were not treated with fungicides, and the resistance frequencies presented here therefore probably underestimate those in farmer's plots treated with chemicals, in which field resistance may occur. We adopted this approach as a means of studying unconstrained resistance dynamics in a population subject to little or no fungicide selection pressure. In the presence of such selection pressure, it would have been difficult to separate out the respective weights of general variation in the population and local selection in treated plots.

Resistance to benzimidazoles (BenR), QoIs (StrR), DMIs (TriR) and SDHIs (CarR) has been monitored and analysed in France since 2004. Our analysis, based on three statistical models, provided a quantitative description of resistance dynamics in time and space.

We found a significant difference in resistance frequencies between the first sampling in spring (T0) and the second sampling in early summer (TNT). The direction of this change within the year depended on the phenotype

considered, and was positively correlated with the between-year dynamics of this phenotype. Plots are continually contaminated with extrinsic ascospores during the cropping season[6], and these ascospores may have contributed to the observed changes in resistance frequency, depending on fungicide selection history within their area of origin. These findings also suggest a possible correlation between fungicide resistance and virulence, as already suggested by Yang *et al.*[59] and Zhan *et al.*[60]. Indeed, as recombination is minimal during the cropping season, host selection, leading to an increase in virulent strains[61] may be accompanied by an increase in the frequency of resistance mutations associated with the such genetic backgrounds. These intra-annual variations were taken into account in our models and should be integrated into measurements of selection pressures.

The emergence of resistance in France was consistent with fungicide use, as resistance emerged with a time lag of about six years after the authorisation of QoIs (1996 to 2002) and SDHIs (2006 to 2012). Regarding resistance to DMIs, the first mutant strains (TriLR phenotypes) were observed in the early 1990s, about ten years after registration. TriMR phenotypes, which first contributed to the erosion of DMIs efficacy, were detected in populations since the early 2000s, almost 20 years after the mode of action registration, whilst TriHR and MDR, associated to the greatest resistance factors emerged only in the late 2000s. In our dataset, resistance dynamics differed between phenotypes in terms of the speed at which resistance developed and its spatial progression, often with differences between northern and southern regions.

Resistance to benzimidazoles (BenR phenotype) is well established in French populations, with a stable inter-annual national mean frequency of about 90%. There seems to be no fitness penalty associated with this resistance, as benzimidazole use is minimal and mostly restricted to *Fusarium* head blight control. BenR frequency was relatively homogeneous over the entire country, with frequencies slightly lower in the Western regions. By contrast, resistance to SDHIs is just emerging and was detected only at a few sites in northern France, and remains negligible. This slow evolution might be the consequence of the recommendation to use only one SDHI spray per season, massively followed by farmers in France.

Resistance to QoIs (StrR phenotype) emerged in the north of France in 2002, one year after it was first reported in the UK and Ireland, and it has since progressively invaded populations (progression of 120-145 km per year), with a clear delimitation each year between the area invaded by resistant strains and a resistance-free area. This is the first time that such a colonization front structure has been quantitatively described for resistance. QoI resistance, which has reached fixation over the entire country since 2009, was found to be increasing in frequency significantly more rapidly than the other resistances, highlighting the high risk of resistance associated with this mode of action. The resistance dynamics for QoIs can be explained by regular selection pressure (two reduced-dose applications of strobilurin associated to a DMI active ingredient, per year, in the early 2000s, in areas with high disease pressure) and the maternal inheritance of the mutation leading to the G143A change (affecting the mitochondrial gene encoding cytochrome *b*), enhancing the transmission of this mutation. This resistance remains fixed in French populations (>95%), possibly due to a very small or non-existent cost of resistance and the non-negligible residual use of QoIs against rusts and *Fusarium* head blight, which may maintain the selection pressure on *Z. tritici* populations for this phenotype. QoI resistance has been reported to occur in a limited number of independent genetic and/or geographic backgrounds, through at least four recurrent mutations in *Z. tritici*[31] in Europe. The multiple emergences of such resistance was recently confirmed in North American populations, in which significant mitochondrial genome bottlenecks were observed[62]. We found that the StrR phenotype spread very quickly from northern to eastern regions in France, but that its progression in the south and west was much slower. This anisotropic dissemination

highlights a preferred direction of migration, from north-western regions to eastern regions, consistent with earlier results showing that gene flow occurs mostly in a west-to-east direction[31]. The lower levels of southward migration may be explained by several factors, including the prevailing direction of the wind, which carries the ascospores[63,64]; lower local selective pressure (only one QoI application yearly) due to smaller epidemics in the south; or the occurrence of large durum wheat areas, on which *Z. tritici* populations have been shown to be genetically different from those on bread wheat populations in analyses based on neutral markers[65]. The propagation speed of 120-145 km/year calculated here is, thus, almost certainly a minimal estimate, because the north-to-south gradient observed in our dataset is not the preferential axis for propagation.

By contrast, DMI resistant strains have been present throughout France since at least 1997[56], but probably for longer, given that DMI selection pressure began in the late 1970s. In UK, progressive decrease in strains sensitivity has been measured over the last 12 years[16]. Quantitative dynamics analysis revealed that some phenotypes were probably counter-selected (negative rates of increase in frequency regularly recorded during the 2004-2017 period, *e.g.* TriLR, associated with deletions or changes in *Cyp51* at codons 459, 460 or 461, or the Y137F or V/C136A variants; TriMR after 2011, associated with the I381V and A379G variants), whereas some others were positively selected (positive rates of increase in frequency regularly recorded, *e.g.* TriMR before 2011; combined changes in TriHR genotypes, including D134G, V/C136A or S524T; MDR, associated with three *mfs1* alleles). This situation is consistent with selective replacement favouring a high diversity of resistant genotypes over the last 30 years. Novel *cyp51* resistance genotypes evolved through *de novo* mutation and intragenic recombination. They are thought to have arisen locally (putatively in UK or Denmark) only once or twice and to have dispersed eastwards across Europe through wind-dispersed ascospores. Regular recombination associated with sexual reproduction, and the continuous use of azoles have led to increases in resistance frequency, particularly for novel genotypes[64]. Similar patterns of diversifying selection and intragenic recombination, leading to the emergence of new genotypes with a selective advantage, have recently been observed in North America[62]. The observed dynamics are consistent with the general gradual erosion of azole efficacy observed in several European countries[16,24,28,29]. Results from a European trial network demonstrated major variations in azole performance across Europe correlated with a clear eastward pattern of decrease in the frequency of all *cyp51* mutations, with the exception of the I381V and A379G variants (TriMR genetic background[18]). Spatial heterogeneity in azole efficacy, correlated with population structure for the frequency of resistant genotypes, was also observed in our dataset at the scale of France. Indeed, from 2005 to 2013, static front structures were detected for TriR6 strains, present mostly in North-East France, and for TriR7 and TriR8 strains, present mostly in the South-West. TriR7 and TriR8 strains were found to be moderately resistant to all DMIs, with the exception of prochloraz[66], potentially accounting for the higher efficacy of this fungicide in this area (Arvalis-Institut du Végétal, unpublished). This spatial structure may result from a founder effect, bringing TriR7 and TriR8 genotypes from northern Europe to the South of France, where they are adapted to the local environment (*e.g.* climatic conditions, cultivars, low prochloraz use). This hypothesis should be explored by performing fitness tests and analysing *cyp51* diversity between strains from northern and southern populations. In more recent French populations from the North and South, contrasting azole efficacies were also observed, with reduced epoxiconazole, prothioconazole, metconazole and tebuconazole efficacy positively associated with higher frequencies of the CYP51 V/C136A and D134G changes, largely present in TriHR genotypes, in the North, compared to the South, in 2016[18,67].

The large amount of data made it possible to perform a fine quantitative and exhaustive

description of resistance dynamics over time and space in this study. To our knowledge, this is the first population study on *Z. tritici* including resistance phenotyping to all unisite modes of action used to control STB, related selection pressures, a large temporal scale (13 years) and a fine spatial coverage (including regional scales for the entire wheat growing area in France), in contrast to previously published snapshot analysis. Our resistance frequency-based approach allowed to phenotype hundreds of populations per year, without the need for laborious strain isolation, thereby providing sufficient spatial coverage annually. The data were collected in a long-term network including diverse stakeholders, demonstrating the power of collective action for monitoring and managing fungicide resistance. Our analysis, based on field data, detected some original spatial cryptic structures and differences in the rate of increase of resistance frequencies between phenotypes and regions, making it possible to describe resistance status in France more precisely than the partial short-term analyses generally performed. This approach should improve regional resistance management. It offers a vision complementary to that provided by the theoretical models of resistance evolution generally proposed, and may also provide empirically validated parameter such as estimates of growth rates. Moreover, the three statistical models used here are not specific to *Z. tritici* and may be considered as generic tools, suitable for use in similar studies of other resistances. The value of this approach for describing resistance dynamics is now well established. Future studies should seek to explain the sources of variation in local resistance evolution, and to identify the extrinsic or intrinsic factors determining resistance dynamics, as these factors may be useful operational levers for resistance management. This could be achieved by developing the DYNAMIC MODEL further. Regional selective pressure (*i.e.* the regional use of fungicides) should be explored, together with demographics (*e.g.* the intensity of local epidemics) and local agronomic and environmental factors. Such improvement to the model would facilitate prediction of the structure of resistant populations year after year, while incorporating any context changes that could affect resistance evolution. Its outputs may help farmers to yearly adapt their spray programs while implementing sustainable local strategies before resistance reaches fixation in population. Indeed, according to the regional resistance dynamics and pesticide use, tailor-made strategies may be recommended, as mixture or alternation regimes may differ in efficacy locally. Like Hicks *et al.*[68], in their long-term nationwide study of herbicide resistance, we conclude that there is a need to use "*an evolutionarily informed approach in a proactive not reactive manner*".

# 5 ACKNOWLEDGEMENTS

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**Supplementary Information 1.** Fungicides used to control STB in France and associated resistance phenomena.

Description and resistance mechanisms are detailed in the following references: BenR[1,2]; TriR[3,4]; StrR[2,5–7]; CarR[8–10]; MDR[3,11–13]. Amino-acid changes were described in the corresponding publications.

| Fungicides | | | | | | | | | | | Resistance¤ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biochemical mode of action§ | | | Chemical structures§ | | | Authorisation in France‡ | | | Codes | | Resistant phenotype abbreviation | Target site resistance | | Non target site resistance | | |
| Main processes | Target site and mode of action | Abbreviation | Main classes | Secondary classes | Active ingredient† | Registration | First year of use | Removal | R4P§ | FRAC† | | Target alteration | Target overexpression | Compensation | Enhanced efflux (MDR) |
| Mitochondrial respiration | Complex II or succinate dehydrogenase. Binding site of ubiquinone involving the subunits SdhB, SdhC and SdhD. | SDHI | Carboxamides | Benzamides | Fluopyram | 2016 | 2017 | - | U-A2a | C2/7 | CarR + | [LR]<br>SdhB: N225I, R265P, T268A/I<br>SdhC: T79N, W80S, N86A/S R151M/S/T, I261S, V166M, T168R<br>SdhD: R47W, I50F, M114F<br><br>[MR-HR]<br>SdhC: T79I, H152R | - | - | +<br>[LR when alone]<br>MFS1 promoter: types I, II or III inserts |
| | | | | Nicotinamides (syn. Pyridine-carboxamides) | Boscalid | 2006 | 2007 | - | | | | | | | |
| | | | | Pyrazole-carboxamides | Benzovindiflupyr | 2016 | 2017 | - | | | | | | | |
| | | | | | Bixafen | 2011 | 2011 | - | | | | | | | |
| | | | | | Penthiopyrad | 2014 | 2014 | - | | | | | | | |
| | | | | | Fluxapyroxad | 2011 | 2011 | - | | | | | | | |
| | Complex III or cytochrome bc1. Binding site of ubiquinone to cytochrome b at the "o" center (proton output) in the heme bl proximal domain | QoI (or QoI-P) | Synthetic strobilurins and analogs | Methoxy-acrylates | Azoxystrobin | 1996 | 1997 | - | U-A5a | C3/11 | StrR + | [LR]<br>Cytb: F129L<br><br>[HR]<br>Cytb: G143A | - | +<br>[LR]<br>AOX over-expression | +<br>[LR when alone]<br>MFS1 promoter: types I, II or III inserts |
| | | | | | Picoxystrobin | 1999 | 2000 | - | | | | | | | |
| | | | | Methoxy-carbamates | Pyraclostrobin | 2000 | 2000 | - | | | | | | | |
| | | | | Oximino-acetates | Kresoxim-methyl | 1996 | 1997 | 2013 | | | | | | | |
| | | | | | Trifloxystrobin | 2001 | 2002 | - | | | | | | | |
| | | | | | Dimoxystrobin | 2009 | 2010 | - | | | | | | | |
| | | | | Oximino-acetamides | Fluoxastrobin | 2006 | 2006 | - | | | | | | | |

| Process | Target | Code§ | Chemical group | Sub-group | Active ingredient | Reg.‡ | Used‡ | Removed‡ | R4P§ | FRAC† | Resistance | +/- | Mutations | Cyp51 promoter | | MFS1 promoter |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sterol biosynthesis | Binding to sterol 14α-demethylase (syn. CYP51) | DMI or SBI-DM | Heterocyclic compounds | Imidazoles | Prochloraz | 1980 | 1980? | - | U-E2 | G1/3 | TriR | + | [LR]<br>Cyp51: one change (*e.g.* Y137F, Y459C/D/H, G460D/S, Y461H/S, ΔY459/G460) or two changes (V136A+Y461H/S or ΔY459/G460)<br><br>[MR]<br>Cyp51: two changes including I381V+Y459S/D/N, Y461H/S or ΔY459/G460<br><br>[HR]<br>Cyp51: three to six changes including those reported for TriLR and TriMR phenotypes, combined with D107V, D134G and/or S524T. | +<br>[LR when alone]<br>Cyp51 promoter: 120 bp or 1000 bp inserts | - | +<br>[LR when alone]<br>MFS1 promoter: types I, II or III inserts |
| | | | | Triazoles | Bromuconazole | 1994 | 1995 | - | | | | | | | | |
| | | | | | Cyproconazole | 1987 | 1987 | - | | | | | | | | |
| | | | | | Difenoconazole | 1988 | 2011 | - | | | | | | | | |
| | | | | | Epoxiconazole | 1992 | 1992 | - | | | | | | | | |
| | | | | | Fenbuconazole | 1991 | 1992 | - | | | | | | | | |
| | | | | | Fluquinconazole | 1997 | 1998 | 2016 | | | | | | | | |
| | | | | | Flusilazole | 1985 | 1986 | 2013 | | | | | | | | |
| | | | | | Flutriafol | 1983 | 1984 | 2015 | | | | | | | | |
| | | | | | Hexaconazole | 1990 | 1990 | 2007 | | | | | | | | |
| | | | | | Metconazole | 1993 | 1994 | - | | | | | | | | |
| | | | | | Propiconazole | 1980 | 1980? | - | | | | | | | | |
| | | | | | Tebuconazole | 1988 | 1989 | - | | | | | | | | |
| | | | | | Tetraconazole | 1991 | 1991? | - | | | | | | | | |
| | | | | | Triadimenol | 1987 | 1988 | 2014 | | | | | | | | |
| | | | | Triazolinethiones | Prothioconazole | 2006 | 2006 | - | | | | | | | | |
| Cell division: microtubules of the mitotic spindle | Binding to β-tubulin | MBC | Benzimidazoles and precursors | Benzimidazoles | Carbendazim | 1972 | 1972? | 2009 | U-K2b | B1/1 | BenR | + | [HR]<br>Tub2: E198A | - | - | - |
| | | | | Thiophanates | Thiophanate-methyl | 1973 | 1980? | - | | | | | | | | |
| Multi-site activity | Fungicide acting non specifically on multiple targets, especially respiratory enzymes | MSI | Dithio-carbamates | Akylene-bis-dithiocarbamates | Mancozeb | 1980 | 1980? | - | U-W11 | M3 | NC | NC | NC | NC | NC | NC |
| | | | | | Maneb | 1988 | 1988? | - | | | | | | | | |
| | | | Chloronitriles | Phthalonitriles | Chlorothalonil | 1980 | 1980? | - | U-W14 | M5 | | | | | | |
| | | | Halogenated alkyl-thio coumpounds | Phthalamides | Folpet | 2003 | 2003 | 2009 | U-W10 | M4 | | | | | | |

? Approximate date.

NC: Not concerned.

§According to the R4P classification (http://www.r4p-inra.fr).

†According to the FRAC classification (http://www.frac.info).

‡Legal authorisation of the active ingredient in France. Year the molecule was registered in France, year it was first used in the field and year it was removed from French registration (http://www.ephy.anses.fr).

[¤]Phenotypes are described in section 2.2 of the main document. [LR]: low resistance levels (<25); [MR]: moderate resistance level (25<RL<100); [HR]: high resistance level (>100).

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**Supplementary Information 2.** *cyp51* genotypes in a collection of pure strains.

Strains were isolated by INRA between 1969 and 2017 (*n*=314). This list is not exhaustive. Other genotypes may exist in populations and have been reported in other studies.

| Main phenotype class[†] | Historical phenotype[†] | Genotype number | Genotype in nomenclature[¤] | 134 | 136 | 137 | 379 | 381 | 459 | 460 | 461 | 524 | Number of changes | cyp51 120 bp | cyp51 1000 bp | mfs1 type 1 | mfs1 type 2 | mfs1 type 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TriS | TriS | G1 | - | D | V | Y | A | I | Y | G | Y | S | 0 | | | | | |
| | | G2 | A5 | D | V | Y | A | I | Y | - | Y | S | 1 | | | | | |
| TriLR | TriR2 | G3 | A1 | D | V | Y | A | I | Y | D | Y | S | 1 | | | | | |
| TriLR | TriR3 | G4 | A6 | D | V | F | A | I | Y | G | Y | S | 1 | | | | | |
| | TriR4 | G5 | D1, D4,D5, E1, E14 | D | V | Y | A | I | - | - | Y | S | 1 | | | | | |
| | | G6 | - | D | V | Y | A | I | H | G | Y | S | 1 | | | | | |
| | | G7 | B8, D24 | D | V | Y | A | I | Y | G | S | S | 1 | | | | | |
| | | G8 | A11, B9, D3 | D | V | Y | A | I | Y | G | H | S | 1 | | | | | |
| | | G9 | E16, F3, F13 | D | C | Y | A | I | - | - | Y | S | 1 | | | | | |
| | | G10 | B10 | D | C | Y | A | I | Y | G | H | S | 1 | | | | | |
| | TriR5 | G11 | C7 | D | A | Y | A | I | Y | G | H | S | 2 | | | | | |
| | | G12 | B2, C6 | D | A | Y | A | I | Y | G | S | S | 2 | | | | | |
| | | G13 | C10, D8, E6, E11, E15, F5 | D | A | Y | A | I | - | - | Y | S | 2 | | | | | |
| TriMR | TriR6 | G14 | B12, C1, C8, C16, D20, D21 | D | V | Y | A | V | Y | G | H | S | 2 | X | | X | | |
| | | G15 | - | D | V | Y | A | V | Y | G | S | S | 2 | | | | | |
| | | G16 | B4, C2 | D | V | Y | A | V | S | G | Y | S | 2 | | | | | |
| | | G17 | B3, C4, D22 | D | V | Y | A | V | D | G | Y | S | 2 | | | | | |
| | | G18 | - | D | V | Y | A | V | N | G | Y | S | 2 | | | | | |
| | TriR7 | G19 | E17, E22, F2, F14 | D | V | Y | A | V | - | - | Y | S | 2 | X | | X | | |
| | TriR8 | G20 | E20, F11, F16, G1, G4, H1, H2, H3, H5, H8 | D | V | Y | G | V | - | - | Y | S | 3 | | | X | | |
| TriHR | TriR9 | G21 | C11, D7 | D | A | Y | A | I | Y | G | S | T | 3 | | | | | |
| | - | G22 | - | D | C | Y | A | I | Y | G | H | T | 2 | | | | | |
| | TriR10 | G23 | C12, D10 | D | A | Y | A | V | Y | G | H | S | 3 | | X | X | | |
| | - | G24 | - | D | A | Y | A | V | Y | G | S | S | 3 | | X | | | X |
| | - | G25 | E5 | D | A | Y | A | V | Y | G | H | T | 4 | | X | X | X | |
| | TriR12 | G26 | E3 | D | A | Y | A | V | Y | G | S | T | 4 | | | | | |
| | TriR11 | G27 | D18, E4 | G | A | Y | A | V | Y | G | H | S | 4 | | | X | X | X |
| | - | G28 | F8, G5 | G | A | Y | A | V | Y | G | H | T | 5 | | | | | X |
| | - | G29 | D13, E7, F4 | D | C | Y | A | V | Y | G | H | T | 3 | | | | | |
| | - | G30 | - | G | V | Y | A | V | Y | G | H | S | 3 | | X | X | | |
| | - | G31 | F7, G7 | D | A | Y | G | V | Y | G | S | T | 5 | | | | | |
| | - | G32 | E9 | G | A | Y | A | V | S | G | Y | S | 4 | | | | | |
| | - | G33 | F1 | D | V | Y | G | V | D | G | Y | T | 4 | | | | | |

| | G | Huf codes | | | | | | | | | | Count | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TriR9 | G34 | E23, F6, G3 | D | A | Y | A | I | - | - | Y | T | 3 | | | | |
| TriRz | G35 | F12 | G | A | Y | A | I | - | - | Y | S | 3 | | | | |
| - | G36 | - | G | C | Y | A | I | - | - | H | S | 3 | | | | |
| - | G37 | G2, H9 | D | V | Y | A | V | - | - | Y | T | 3 | | | | |
| - | G38 | - | G | V | Y | A | V | - | - | H | S | 4 | X | | | |
| - | G39 | H4, I1 | D | A | Y | G | V | - | - | Y | T | 5 | | | | |
| - | G40 | F9, H6, I3 | D | C | Y | G | V | - | - | Y | T | 4 | | | | |
| - | G41 | - | G | C | Y | G | V | - | - | H | T | 6 | | | X | |
| - | G42 | - | G | V | Y | G | V | - | - | H | S | 5 | | | X | |

[†]Nomenclature described in Leroux *et al.*[1] and Leroux & Walker[2].

[‡]Numbers indicate codons affected by mutation or deletion within the cyp51 sequences of the strains of the collection. Letters indicate amino acids according to standard nomenclature. For the sake of simplicity, only codons relevant for azole resistance are reported, according to Cools *et al.*[3] and Cools *et al.*[4] with the exception of changes affecting the DYGYG motif. The L50S, D107V, N178S, S188N, S208T, S259F, N284H, H303Y, A311G, G312A, A410T, G412A, G476S, V490L, N504K, G510C and/or N513K variants are not related to azole resistance but are regularly reported in most genotypes, and would therefore greatly increase the total number of genotypes if reported.

[¤]Nomenclature described in Huf *et al.*[5]. As more codons are considered in this nomenclature, several codes were possible to match with our genotype numbers.

[§]Description of additional resistance mechanisms possibly affecting strain susceptibility to azoles. Two types of insertion have been described in the promoter sequence of *cyp51* and the shortest one has been shown to lead to *cyp51* overexpression[6,7]. Three insertions (types 1-3) in the promoter sequence of *mfs1* have been shown to induce overexpression, causing multidrug resistance (enhanced efflux from the membrane transporter MFS1,[8,9]). 'X' indicates that the insertion was regularly found in the corresponding *cyp51* background, as the three resistance mechanisms are independent. Some combinations may be missing.

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**Supplementary Information 3.1.** ANOVA MODEL

**Model:**

The observation $Y_{ijklm}$ of the $m^{\text{th}}$ frequency observed for phenotype $i$, year $j$, region $k$ and sampling date $l$ was modelled using a zero-and-one inflated binomial model:

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

$$logit(p_{ijklm}) = \mu_i + \alpha_{ij} + \beta_{ik} + \gamma_{ijk} + \delta_{il} + \varepsilon_{ijklm}$$

$$\text{where } \varepsilon_{ijklm} \underset{ind.}{\sim} \mathcal{N}(0, \ \sigma_i^2)$$

With,

$\mu_i$: Intercept, or interannual and national mean frequency for phenotype $i$

$\alpha_{ij}$: Effect of the $j^{\text{th}}$ year for phenotype $i$

$\beta_{ik}$: Effect of the $k^{\text{th}}$ region for phenotype $i$

$\gamma_{ijk}$: Interaction effect between the $j^{\text{th}}$ year and the $k^{\text{th}}$ region for phenotype $i$

$\delta_{il}$: Effect of the $l^{\text{th}}$ sampling date for phenotype $i$

$\sigma_i$: Overdispersion parameter for phenotype $i$

**R Script (JAGS):**

```
model{
        #
        # Likelihood
        #
        for(i in 1:n){
                y[i] ~ dbin(P[i], 100)
        #
                P[i] <- p0[i] * 1.0E-10 +  pp[i] * p[i]  +  p100[i] * (1-1.0E-10)
        #
                p0[i]   <- equals(inflated[i, phenotype[i]], 1)
                pp[i]   <- equals(inflated[i, phenotype[i]], 2)
                p100[i] <- equals(inflated[i, phenotype[i]], 3)
                inflated[i, phenotype[i]] ~ dcat(pi[, phenotype[i]])
        #
                p[i] <- exp(eta[i])/(1 + exp(eta[i]))
        #
                eta[i] ~ dnorm(mu[i], Phenotype_tau[phenotype[i]])
                mu[i] <- Phenotype.YearXPhenotype.RegionXYearXPhenotype[region[i], year[i],
phenotype[i]] +
                        RegionXPhenotype[region[i], phenotype[i]] +
                        Sampling.dateXPhenotype[sampling.date[i], phenotype[i]]
        }
        #
        # Inflated parameters
        #
        for(i in 1:n_phenotype){
                pi[1:3, i] ~ ddirch(alpha[])
        }
        #
        for(i in 1:3){
```

```
                alpha[i] <- 1/2
        }
        #
        # Priors
        #
        for(i in 1:n_phenotype){
                Phenotype[i] ~ dnorm(0, 1.0E-6)
        #
                for(j in 1:n_year){
                        Phenotype.YearXPhenotype[j, i] ~ dnorm(Phenotype[i],
invVar.YearXPhenotype[i])
                        YearXPhenotype[j, i] <- Phenotype.YearXPhenotype[j, i] - Phenotype[i]
                #
                        for(k in n_region){
                                Phenotype.YearXPhenotype.RegionXYearXPhenotype[k, j, i] ~
dnorm(Phenotype.YearXPhenotype[j, i], invVar.RegionXYearXPhenotype[k, i])
                                RegionXYearXPhenotype[k, j, i] <-
Phenotype.YearXPhenotype.RegionXYearXPhenotype[k, j, i] - Phenotype.YearXPhenotype[j, i]
                        }
                }
        #
                for(k in 1:n_region){
                        RegionXPhenotype[k, i] ~ dnorm(0, invVar.RegionXPhenotype[i])
                }
        #
                Sampling.dateXPhenotype[1, i] ~ dnorm(0, 1.0E-6)
        #
        #
        #
                invVar.YearXPhenotype[i] <- 1/(Sigma.YearXPhenotype[i]*Sigma.YearXPhenotype[i])
                Sigma.YearXPhenotype[i] ~ dlnorm(0, 1)
        #
                invVar.RegionXPhenotype[i] <-
1/(Sigma.RegionXPhenotype[i]*Sigma.RegionXPhenotype[i])
                Sigma.RegionXPhenotype[i] ~ dlnorm(0, 1)
        #
                for(k in 1:n_region){
                        invVar.RegionXYearXPhenotype[k, i] <- 1/(Sigma.RegionXYearXPhenotype[k,
i]*Sigma.RegionXYearXPhenotype[k, i])
                        Sigma.RegionXYearXPhenotype[k, i] ~ dlnorm(0, 1)
                }
        #
                Phenotype_tau[i] <- 1/(Sigma.Phenotype_tau[i]*Sigma.Phenotype_tau[i])
                Sigma.Phenotype_tau[i] ~ dlnorm(0, 1)
        }
        #
        # Constraints
        #
        for(i in 1:n_phenotype){
                Sampling.dateXPhenotype[2, i] <- 0
        }
}
```

**Supplementary Information 3.2.** DYNAMIC MODEL

**Model:**

The observation $Y_{ijklm}$ of the $m^{\text{th}}$ frequency observed for phenotype $i$, year $j$, region $k$ and sampling date $l$ was modelled using a zero-and-one inflated binomial model:

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

The probability $p_{ijklm}$ was modelled using a model of selection in haploid organisms found in Hartl and Clark[1]. For an observation (fixed $m$), of a given resistance phenotype (fixed $i$) and sampling date (fixed $l$) in a given region (fixed $k$), we note:
- $R$ the phenotype resistant to a given fungicide and $S$ its associated sensitive phenotype.
- $\omega_R$ the fitness (*i.e.* ability to survive and reproduce in an environment) of $R$, and $\omega_S$ the fitness of $S$. Then, $\omega = \omega_R/\omega_S$ is the relative fitness of R against S.

We focus now on $p_j$ the proportion of $R$ in the population year $j$. We also have $q_j = (1 - p_j)$ the associated proportion of $S$ in the population year $j$.

We have:

| | Time | R | S |
|---|---|---|---|
| Proportion before selection | $j-1$ | $p_{j-1}$ | $q_{j-1}$ |
| Proportion after selection | $j$ | $p_j = \frac{\omega * p_{j-1}}{\omega * p_{j-1} + q_{j-1}}$ | $q_j = \frac{q_{j-1}}{\omega * p_{j-1} + q_{j-1}}$ |

Then, we can write: $\frac{p_j}{q_j} = \omega * \frac{p_{j-1}}{q_{j-1}} = \omega^2 * \frac{p_{j-2}}{q_{j-2}} = ... = \omega^{j-1} * \frac{p_1}{q_1}$

Or: $ln(\frac{p_j}{1-p_j}) = (j - 1) * ln(\omega) + ln(\frac{p_1}{q_1})$

We obtain: (1)   $logit(p_j) = \mu + \beta * (j - 1)$

Where $\mu = ln(\frac{p_1}{q_1})$ quantifies the initial proportion (at $j = 1$) of $R$ , and $\beta = ln(\omega)$ quantifies the relative fitness of $R$ against $S$.
The frequency of $R$ tends to increase if its fitness ($\omega_R$) is superior to that fitness of $S$ ($\omega_S$), thus if $\omega > 1$, thus if $\beta > 0$.

Now, if we want to generalise equation (1) to several observations ($m$) of different phenotypes ($i$) in different regions ($k$), we can write:

(2)   $logit(p_{ijkm}) = \mu_i + \alpha_{ik} + (\beta_i + \gamma_{ik}) * (j - 1)$

With,
$\mu_i$: Intercept, or initial national frequency ($j = 1$) for phenotype $i$
$\alpha_{ik}$: Effect of the $k^{\text{th}}$ region on initial national frequency for phenotype $i$
$\beta_i$: National growth rate for phenotype $i$
$\gamma_{ik}$: Effect of the $k^{\text{th}}$ region on national growth rate for phenotype $i$

Finally, model (2) was extended to include effects of sampling dates and overdispersion:

(3)   $logit(p_{ijklm}) = \mu_i + \alpha_{ik} + (\beta_i + \gamma_{ik}) * (j - 1) + \delta_{il} + \varepsilon_{ijklm}$

$$\text{where } \varepsilon_{ijklm} \underset{ind.}{\sim} \mathcal{N}(0, \ \sigma_i^2)$$

With,
$\delta_{il}$: Effect of the $l^{\text{th}}$ sampling date for phenotype $i$
$\sigma_i$: Overdispersion parameter for phenotype $i$

**R script (JAGS):**

```
model{
        #
        # Likelihood
        #
        for(i in 1:n){
                y[i] ~ dbin(P[i], 100)
        #
                P[i] <- p0[i] * 1.0E-10  +  pp[i] * p[i]  +  p100[i] * (1-1.0E-10)
        #
                p0[i]   <- equals(inflated[i, phenotype[i]], 1)
                pp[i]   <- equals(inflated[i, phenotype[i]], 2)
                p100[i] <- equals(inflated[i, phenotype[i]], 3)
                inflated[i, phenotype[i]] ~ dcat(pi[, phenotype[i]])
        #
                p[i] <- exp(eta[i])/(1 + exp(eta[i]))
        #
                eta[i] ~ dnorm(mu[i], Phenotype_tau[phenotype[i]])
                mu[i] <- Phenotype.RegionXPhenotype_T0[region[i], phenotype[i]] +
                        Phenotype.RegionXPhenotype[region[i], phenotype[i]]*time[i] +
                        Sampling.dateXPhenotype[sampling.date[i], phenotype[i]]
        }
        #
        # Inflated parameters
        #
        for(i in 1:n_phenotype){
                pi[1:3, i] ~ ddirch(alpha[])
        }
        #
        for(i in 1:3){
                alpha[i] <- 1/2
        }
        #
        # Priors
        #
        for(i in 1:n_phenotype){
                Phenotype_T0[i] ~ dnorm(0, 1.0E-6)
        #
                Phenotype[i] ~ dnorm(0, 1.0E-6)
        #
                for(j in 1:n_region){
                        Phenotype.RegionXPhenotype_T0[j, i] ~ dnorm(Phenotype_T0[i],
invVar.RegionXPhenotype_T0[i])
                        RegionXPhenotype_T0[j, i] <- Phenotype.RegionXPhenotype_T0[j, i] -
Phenotype_T0[i]
                #
                        Phenotype.RegionXPhenotype[j, i] ~ dnorm(Phenotype[i],
invVar.RegionXPhenotype[i])
                        RegionXPhenotype[j, i] <- Phenotype.RegionXPhenotype[j, i] -
Phenotype[i]
                }
        #
                Sampling.dateXPhenotype[1, i] ~ dnorm(0, 1.0E-6)
        #
        #
        #
                invVar.RegionXPhenotype_T0[i] <-
1/(Sigma.RegionXPhenotype_T0[i]*Sigma.RegionXPhenotype_T0[i])
                Sigma.RegionXPhenotype_T0[i] ~ dlnorm(0, 1)
        #
                invVar.RegionXPhenotype[i] <-
1/(Sigma.RegionXPhenotype[i]*Sigma.RegionXPhenotype[i])
                Sigma.RegionXPhenotype[i] ~ dlnorm(0, 1)
        #
                Phenotype_tau[i] <- 1/(Sigma.Phenotype_tau[i]*Sigma.Phenotype_tau[i])
                Sigma.Phenotype_tau[i] ~ dlnorm(0, 1)
        }
        #
        # Constraints
        #
        for(i in 1:n_phenotype){
                Sampling.dateXPhenotype[2, i] <- 0
        }
}
```

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**Supplementary Information 4.** Estimates for the ANOVA MODEL, for year, region and sampling date effects.

Estimates of interactions between region and year are not shown because the factor was not significant for all phenotypes. Estimates are given on a logit scale. Disregarding the value of the Year:Region interaction parameter, frequencies can be recovered (in %) by calculating the inverse logit of estimates, weighting by the estimated proportions of 0 and 100 ($\pi_0$ and $\pi_{100}$, respectively), and multiplying the value obtained by 100. For instance, the frequency estimated for StrR in 2011, in PCH at the T0 sampling time was: $[0.02*0 + (1-0.02-0.29) *\text{logit}^{-1}(1.98+1.61-0.5-0.53)*)*(1-0.29-0.02)0.69 + 1*0.29*1 + 0*0.02]*] * 100 = 93\%$. Empty cells indicate that no data were available for estimation. Factors tests *P*-values are indicated in bold and italic. *P*-value thresholds: "·" ($P < 0.1$), "*" ($P < 0.05$), "**" ($P < 0.025$), "***" ($P < 0.001$).

| Factor | Parameter | Phenotype | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | BenR | StrR | TriLR | TriR2-TriR4 | TriR5 | TriMR | TriR6 | TriR7-TriR8 | TriHR | MDR |
| | Inter-annual national mean | 1.78*** | 1.98* | -1.97*** | -2.1*** | -2.52*** | 1.19*** | 0.31 | -1.32*** | -1.87*** | -1.99*** |
| Year | | **0.894** | **0***** | **0***** | **0.065 ·** | **0***** | **0***** | **0***** | **0***** | **0.034*** | **0.972** |
| | 2004 | | -3.04** | | | | | | | | |
| | 2005 | | -1.93* | 1.7*** | | | -0.83** | | | | |
| | 2006 | | -0.89 | 0.99* | 0.31* | 0.45 | -0.25 | -0.15 | -0.07 | | |
| | 2007 | -0.2 | -0.57 | 0.76 · | 0.27 · | 0.72* | -0.05 | -0.15 | 0.16 | | |
| | 2008 | -0.14 | -0.56 | 0.76 · | 0.31* | 0.55* | -0.03 | -0.15 | 0.09 | | |
| | 2009 | 0.14 | -0.34 | 0.28 | -0.03 | 0.47 · | 0.42* | 0.5* | -0.35 | | |
| | 2010 | 0.03 | 0.94 | 0.13 | -0.2 | 0.23 | 0.4* | 0.5* | -0.36 | -1.43** | |
| | 2011 | 0 | 1.61 · | -0.06 | -0.17 | -0.04 | 0.5* | 0.56* | -0.19 | -0.58 | |
| | 2012 | 0.24 | 4.84*** | -0.04 | -0.04 | -0.29 | 0.17 | 0.35 | -0.74** | -0.42 | |
| | 2013 | 0.16 | | 0.18 | 0.18 | -0.1 | 0.04 | -0.55* | 0.52* | 0.05 | -0.18 |
| | 2014 | 0.03 | | -0.54 | -0.32 | -0.71 · | 0.15 | 0.45 | -0.18 | 0.38 | -0.08 |
| | 2015 | -0.18 | | -1.46** | -0.3 | -1.26** | 0.37 | 0.39 | 0.34 | 0.13 | 0.22 |
| | 2016 | 0.03 | | -2.87*** | | | -0.55* | -0.14 | -0.54 | 1.07* | 0.1 |
| | 2017 | -0.09 | | | | | -0.41 | -1.57*** | 1.37*** | 0.8 · | -0.05 |
| Region | | **0***** | **0.224** | **0.004**** | **1** | **0.018*** | **0.99** | **0.01*** | **0***** | **1** | **1** |
| | NPC | 0.58* | 1.06* | -0.49* | -0.06 | -0.41 | 0.17 | 0.54* | -0.64* | 0.34 | -0.02 |
| | PIC | 0.56** | 0.47 | -0.59* | -0.18 | -0.6* | 0.16 | 0.4 · | -0.52 · | 0.27 | 0 |
| | HNO | 0.61 · | 0.79* | -0.3 | -0.06 | 0.02 | 0.15 | 0.44 · | -0.43 | 0.01 | |
| | BNO | 0.18 | 0.23 | 0.08 | 0.09 | -0.07 | -0.14 | 0.18 | -0.66* | 0.39 | |
| | LOR | 0.35 | -0.02 | 0.15 | 0 | -0.17 | -0.03 | 0.34 | -0.46 | -0.19 | |
| | CHA | 0.25 | 0.19 | -0.05 | -0.04 | -0.19 | 0.05 | 0.29 | -0.45 · | 0.15 | -0.03 |
| | IDF | 0.27 | 0.43 | -0.1 | -0.03 | 0.03 | 0.07 | 0.53* | -0.46 | -0.27 | -0.05 |
| | BRE | -0.67** | 0.21 | -0.27 | -0.02 | -0.33 · | 0.07 | -0.23 | 0.42 | 0.12 | |
| | CEN | 0.03 | 0.4 | 0.34* | 0.02 | 0.38 · | -0.07 | -0.03 | -0.16 | -0.35 | 0.01 |
| | PDL | -0.48 · | -1.08* | 0.28 | 0.02 | 0.6* | -0.17 | -0.37 · | 0.31 | -0.05 | 0.09 |
| | BOU | 0.05 | -0.22 | 0.17 | 0.05 | 0.15 | -0.02 | -0.04 | -0.14 | | |
| | PCH | -0.2 | -0.5 | 0.57** | -0.04 | 0.66** | -0.22 | -0.45 | 0.63* | -0.13 | |
| | AUV | 0.04 | -0.09 | 0.23 | 0.09 | 0.17 | -0.17 | -0.08 | -0.22 | 0.15 | |
| | RAL | -0.32 | -0.1 | 0.14 | 0.04 | -0.03 | -0.03 | 0.18 | -0.07 | -0.33 | |
| | AQU | -0.91** | -1.19 · | 0.06 | 0.07 | 0.1 | 0.03 | -0.64 · | 0.94* | -0.12 | |
| | MPY | -0.33 | -0.53 | -0.21 | 0.06 | -0.27 | 0.18 | -1.1 · | 1.88** | | |
| Year:Region | | **1** | **0.661** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** |
| Sampling date | TNT-T0 | 0.04 | 0.53*** | -0.21** | -0.25** | -0.06 | 0.2** | 0.39*** | -0.11 | 0.61 | |
| | $\pi_0$ | 0 | 0.02 | 0.1 | 0.3 | 0.4 | 0 | 0.05 | 0.17 | 0.47 | 0.61 |
| | $\pi_{100}$ | 0.3 | 0.29 | 0 | 0 | 0 | 0.07 | 0.03 | 0 | 0 | 0 |
| | $R^2$ | 0.45 | 0.84 | 0.59 | 0.41 | 0.53 | 0.42 | 0.64 | 0.58 | 0.47 | 0.6 |

**Supplementary Information 5.** Estimates for the DYNAMIC MODEL.

Regional adjustments of the initial national frequency were also estimated but are not shown here. Estimates are given on a logit scale. Initial frequency refers to frequencies observed in the column "First year" of **Table 1**. To recover frequencies (in %) the inverse logit of estimates must be calculated, weighted by the estimated proportion of 0 and 100 ($\pi_0$ and $\pi_{100}$, respectively), and the value obtained should then be multiplied by 100. For instance, for the estimated frequency of StrR in 2011 in PCH at the T0 sampling: [logit$^{-1}$(-1.04+(0.68+0.34)*7-0.58)*0.69 + 1*0.3 + 0*0.02]*100 = 98%. Empty cells indicate that no data were available for the estimation. *P*-value thresholds: "·" ($P < 0.1$), "*" ($P < 0.05$), "**" ($P < 0.025$), "***" ($P < 0.001$).

| Factor | Parameter | Phenotype | | | | | | | | | | |
| | | BenR | StrR | TriLR | TriR2-TriR4 | TriR5 | TriMR ≤2011 | TriMR ≥2011 | TriR6 | TriR7-TriR8 | TriHR | MDR |
| | Initial national frequency | 1.65*** | -1.04* | -0.59** | -1.62*** | -1.79*** | 0.59** | 1.71*** | 0.29 | -1.43*** | -3.11*** | -2.05*** |
| | National growth rate | 0.03 | 0.68*** | -0.21*** | -0.1*** | -0.14*** | 0.22*** | -0.17** | 0 | 0.03 | 0.37*** | 0.09 |
| Regional adjustment | NPC | 0 | -0.26· | 0.12* | 0.04 | -0.01 | -0.13· | 0.03 | -0.06 | -0.03 | -0.04 | -0.02 |
| | PIC | -0.01 | -0.35*** | 0.01 | 0.01 | -0.06 | -0.13* | -0.04 | -0.08· | -0.03 | -0.07 | -0.04 |
| | HNO | 0.02 | -0.05 | 0.06 | 0.04 | 0.02 | -0.09 | -0.04 | 0.05 | -0.07 | -0.01 | |
| | BNO | 0 | -0.02 | -0.04 | -0.02 | -0.02 | 0 | -0.02 | -0.01 | -0.12 | -0.05 | |
| | LOR | 0.01 | -0.13 | -0.05 | 0 | 0.01 | 0.1 | 0.02 | 0 | 0.04 | 0 | |
| | CHA | 0.01 | -0.34* | -0.02 | 0.01 | 0 | 0.12· | -0.13· | -0.13** | 0.04 | 0.09 | -0.02 |
| | IDF | 0 | -0.05 | -0.07 | -0.01 | 0.02 | 0.03 | 0 | 0.08 | -0.16* | -0.01 | 0 |
| | BRE | 0.02 | 0.17 | 0.06 | 0.03 | 0.02 | -0.07 | -0.08 | -0.04 | -0.06 | 0.06 | |
| | CEN | -0.07* | -0.1 | -0.01 | 0.01 | 0 | 0.01 | 0.06 | 0 | 0.03 | 0.05 | -0.04 |
| | PDL | 0.07* | 0.16 | -0.02 | 0 | -0.02 | 0.06 | -0.03 | 0.09· | -0.02 | 0.03 | 0.11 |
| | BOU | 0.03 | -0.15 | 0.03 | 0 | 0.02 | -0.08 | 0.1 | -0.05 | 0.14* | | |
| | PCH | -0.04 | 0.34* | -0.02 | -0.02 | -0.01 | 0.09 | 0.05 | 0.13* | 0.03 | -0.13 | |
| | AUV | 0.01 | -0.1 | 0.07 | 0.02 | 0.04 | -0.08 | -0.08 | 0.01 | -0.07 | 0.07 | |
| | RAL | -0.02 | 0.11 | -0.13* | -0.05 | -0.03 | 0.19* | 0.04 | 0.16* | 0.02 | 0.01 | |
| | AQU | -0.02 | 0.2 | 0.05 | -0.02 | 0.02 | -0.05 | 0.04 | 0.01 | 0.02 | 0.01 | |
| | MPY | -0.02 | 0.53** | -0.03 | -0.02 | 0 | 0.01 | 0.09 | -0.15* | 0.22*** | | |
| Sampling date | TNT-T0 | 0.06 | 0.58*** | -0.08 | -0.16* | -0.12· | 0.16* | 0.07 | 0.29*** | -0.13 | 0.58· | |
| | $\pi_0$ | 0 | 0.02 | 0.12 | 0.3 | 0.41 | 0 | 0 | 0.05 | 0.17 | 0.49 | 0.61 |
| | $\pi_{100}$ | 0.3 | 0.3 | 0 | 0 | 0 | 0.06 | 0.09 | 0.03 | 0 | 0 | 0 |
| | R² | 0.4 | 0.86 | 0.51 | 0.33 | 0.51 | 0.45 | 0.25 | 0.53 | 0.48 | 0.52 | 0.49 |

**Supplementary Information 6.1.** Dynamics of resistance to antimicrotubule agents (BenR phenotype) in *Z. tritici* in France between 2007 and 2017. Output of the SPATIAL MODEL. Colours correspond to extrapolated resistance frequencies.
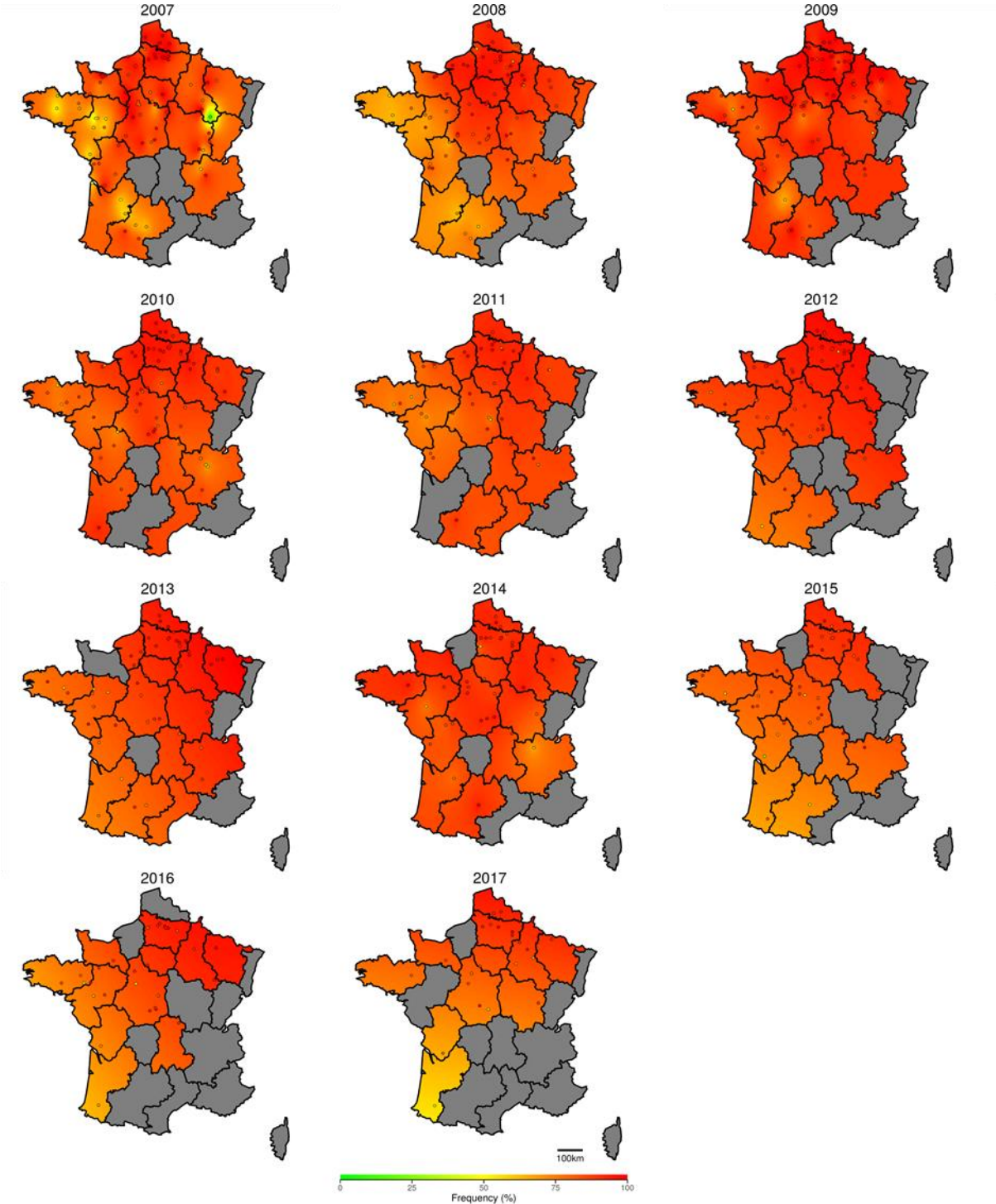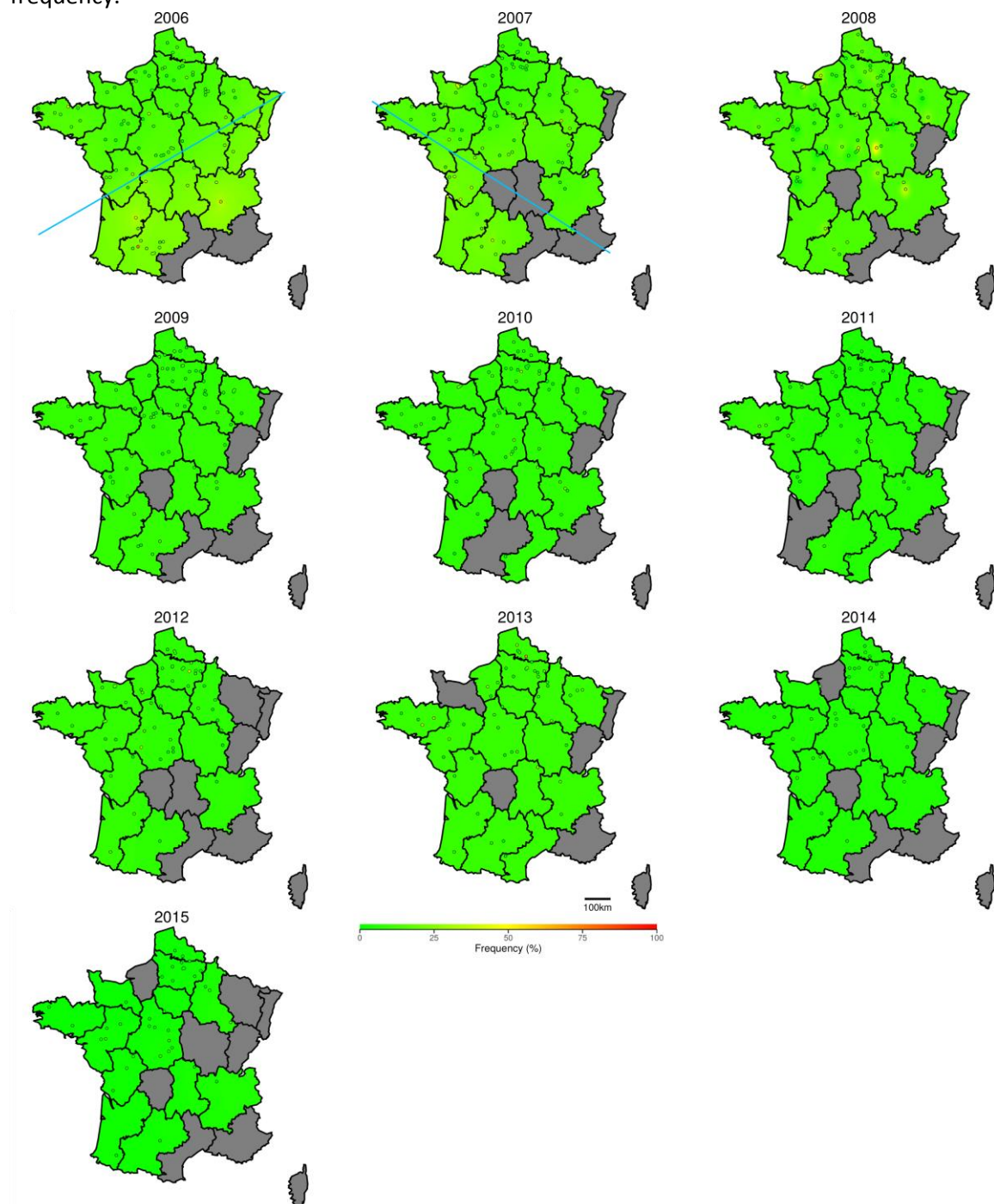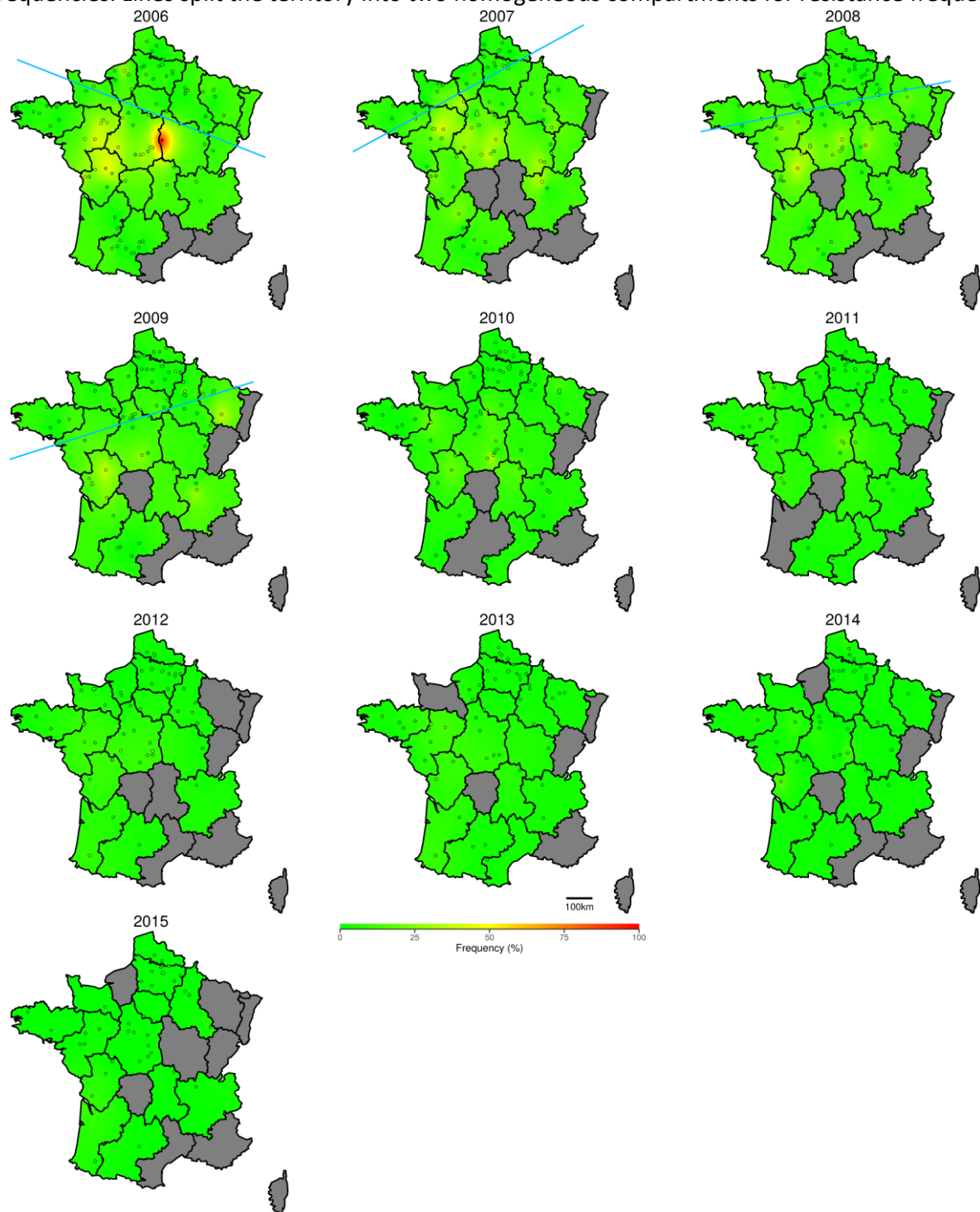
**Supplementary Information 6.2.** Dynamics of resistance to QoIs (StrR phenotype) in *Z. tritici* in France between 2004 and 2012. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.
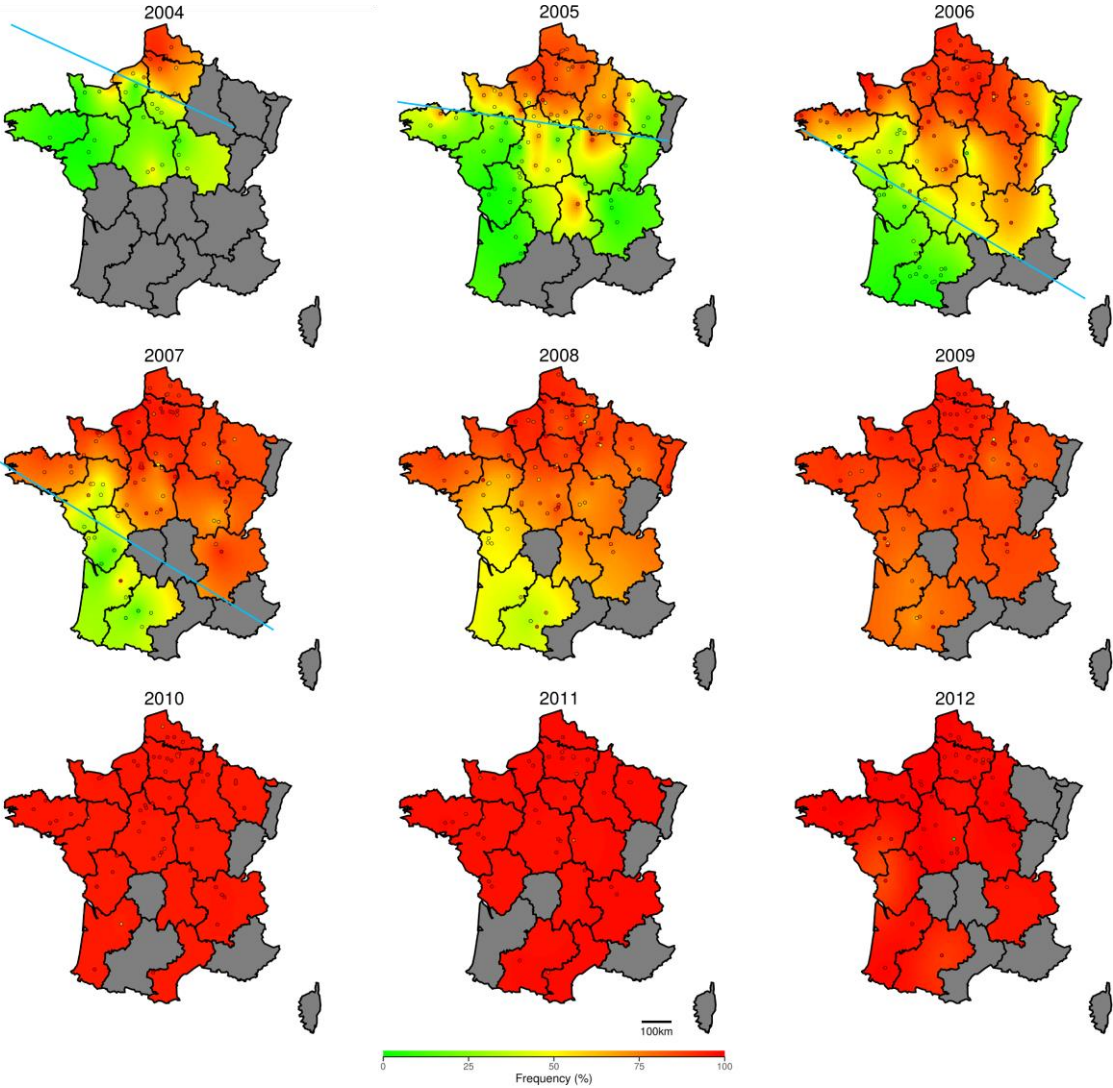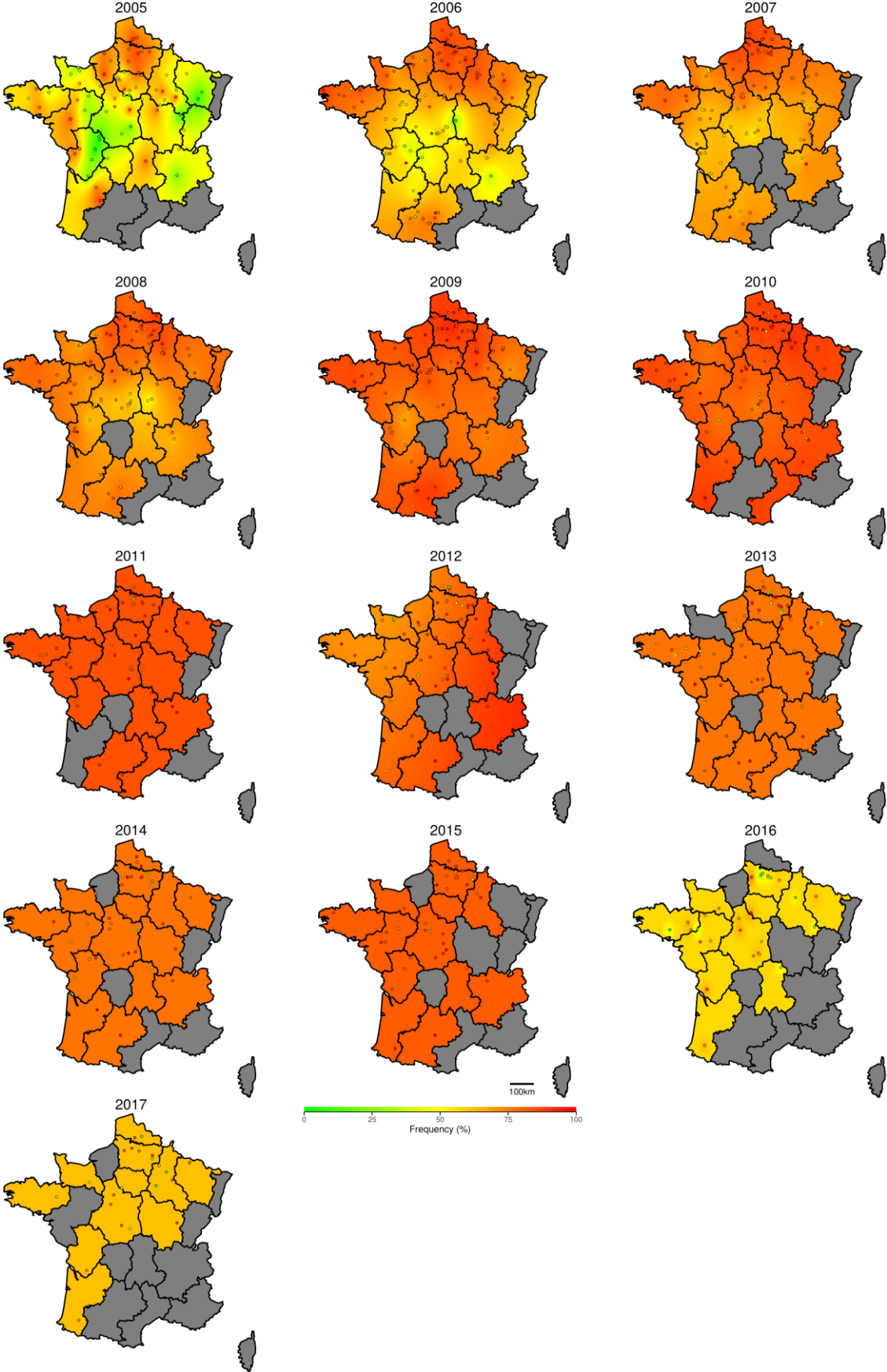
**Supplementary Information 6.3.** Dynamics of resistance to DMIs (TriLR phenotypes) in *Z. tritici* in France between 2005 and 2016. Output of the SPATIAL MODEL. Colours indicated extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.
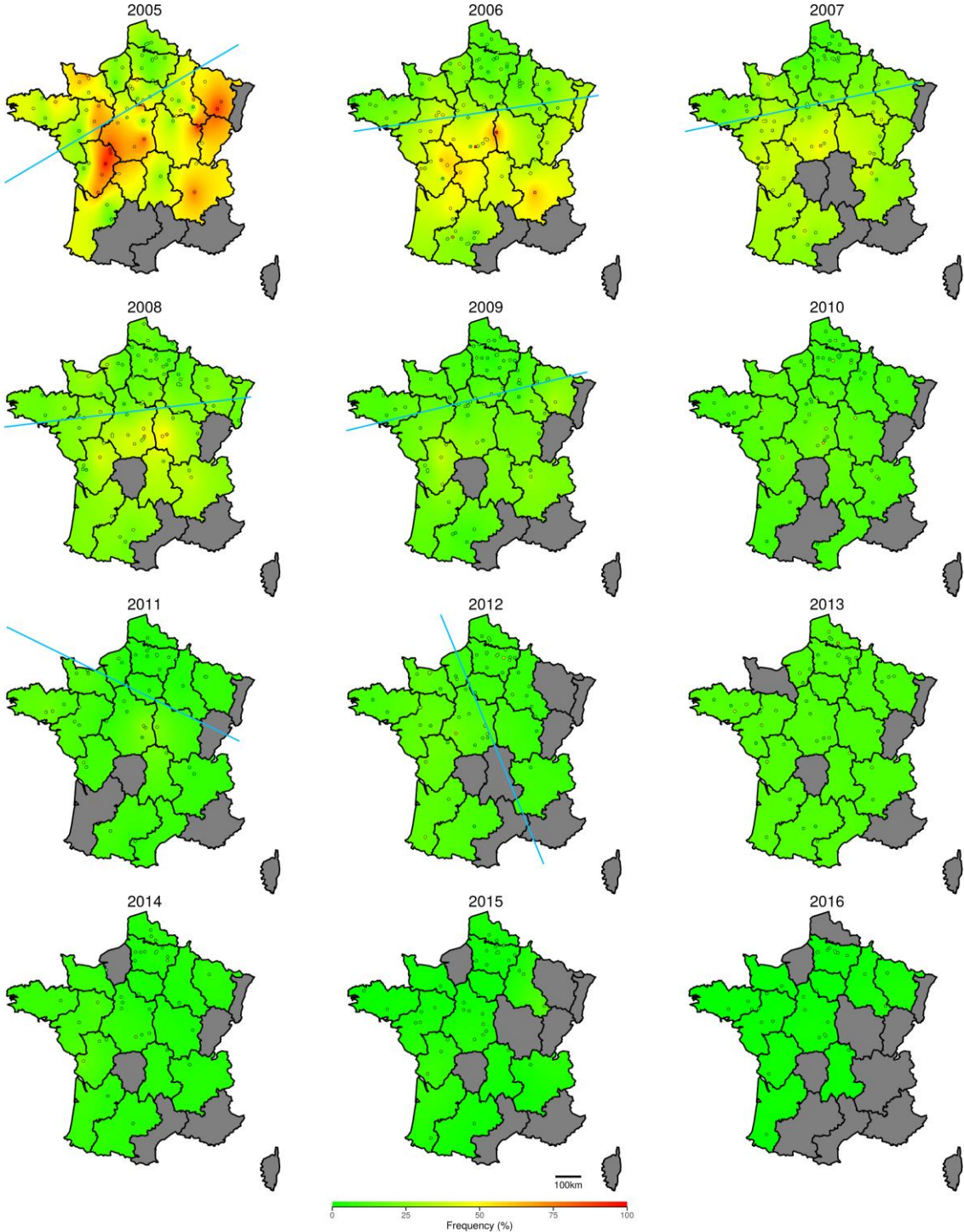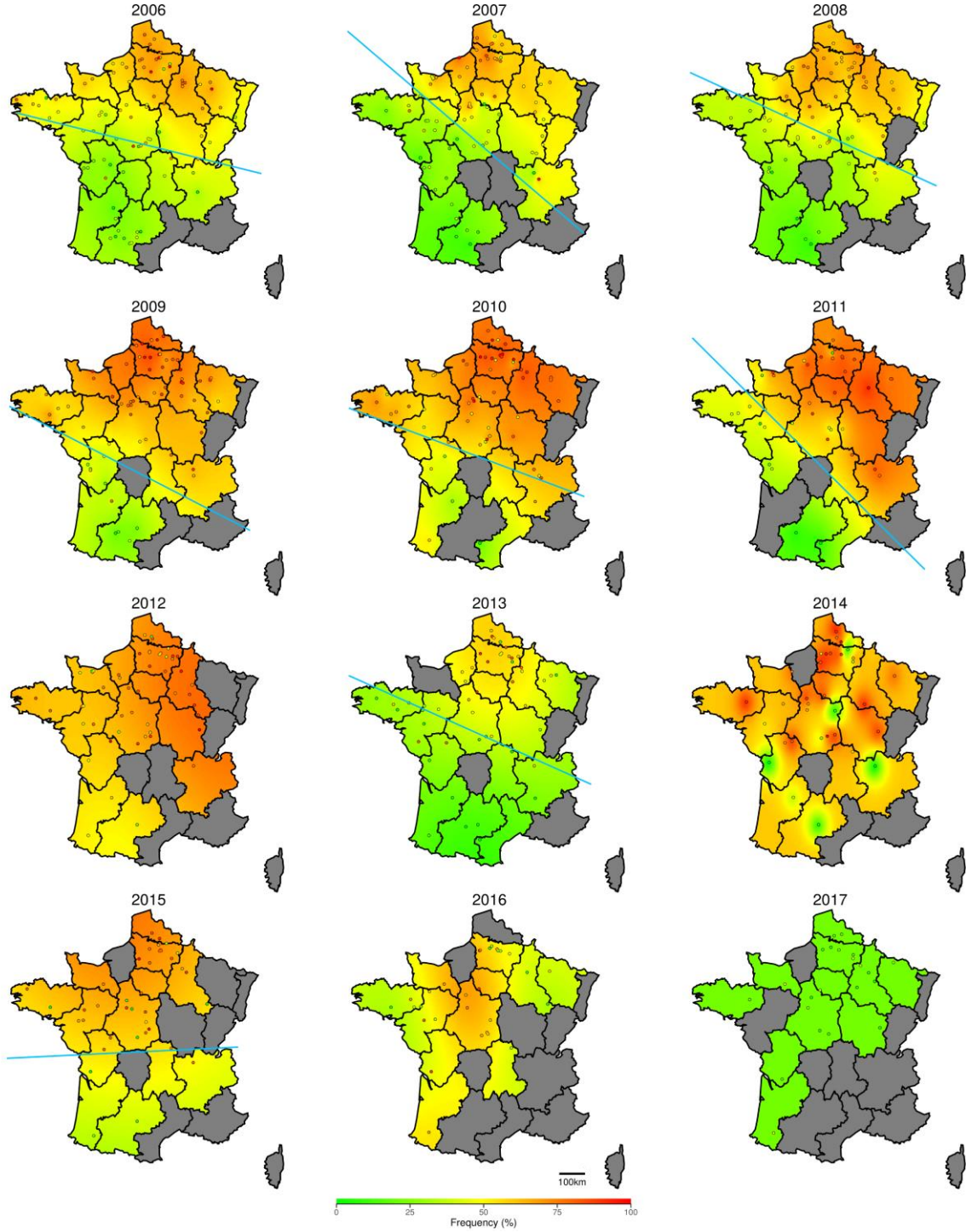
**Supplementary Information 6.4.** Dynamics of resistance to DMIs (TriR2-TriR4 phenotypes) in *Z. tritici* in France between 2006 and 2015. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.
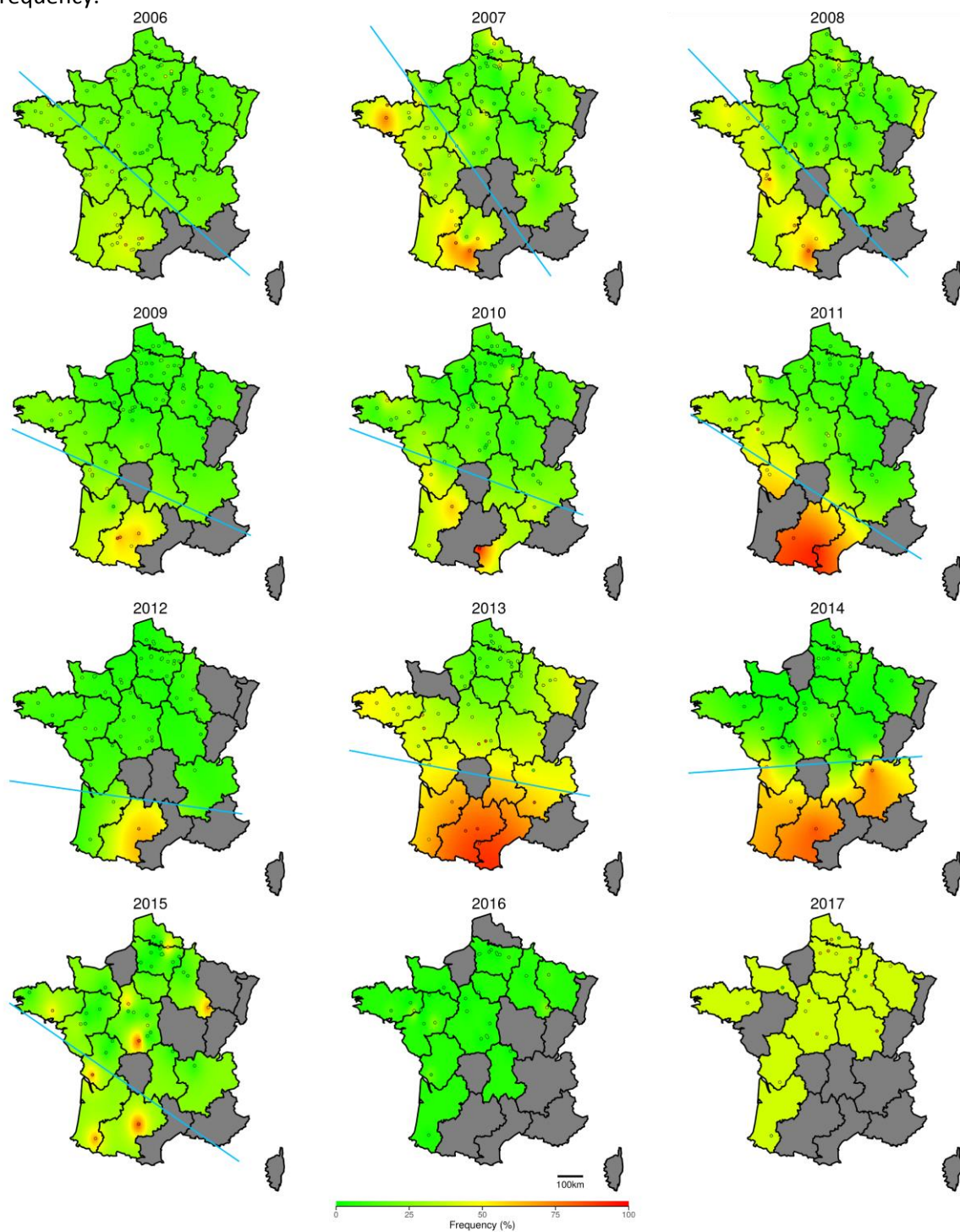
**Supplementary Information 6.5.** Dynamics of resistance to DMIs (TriR5 phenotype) in *Z. tritici* in France between 2006 and 2015. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.

**Supplementary Information 6.6.** Dynamics of resistance to DMIs (TriMR phenotypes) in *Z. tritici* in France between 2005 and 2017. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies.
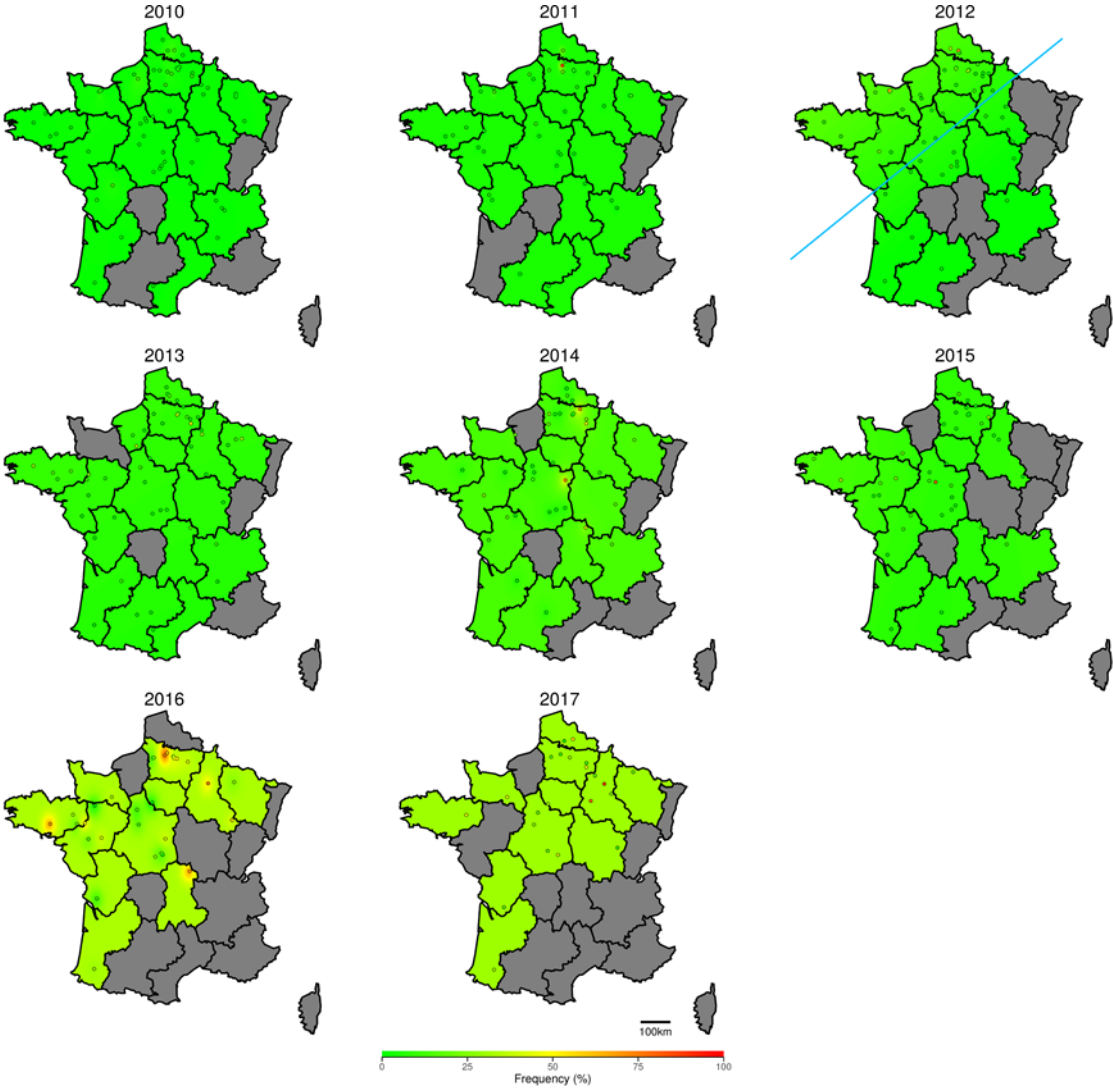
**Supplementary Information 6.7.** Dynamics of resistance to DMIs (TriR6 phenotype) in *Z. tritici* in France between 2006 and 2017. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.

**Supplementary Information 6.8.** Dynamics of resistance to DMIs (TriR7-TriR8 phenotypes) in *Z. tritici* in France between 2006 and 2017. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.

**Supplementary Information 6.9.** Dynamics of resistance to DMIs (TriHR phenotypes) in *Z. tritici* in France between 2010 and 2017. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.

**Supplementary Information 6.10.** Dynamics of resistance to DMIs, QoIs and SDHIs (MDR phenotypes) in *Z. tritici* in France between 2013 and 2017. Output of the SPATIAL MODEL. Colours indicate extrapolated resistance frequencies. Lines split the territory into two homogeneous compartments for resistance frequency.