Research Article

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Spatio-temporal distribution of DMI and SDHI fungicide resistance of *Zymoseptoria tritici* throughout Europe based on frequencies of key target-site alterations

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Abstract

BACKGROUND: Over the past decade, demethylation inhibitor (DMI) and succinate dehydrogenase inhibitor (SDHI) fungicides have been extensively used to control to septoria tritici blotch, caused by *Zymoseptoria tritici* on wheat. This has led to the development and selection of alterations in the target-site enzymes (CYP51 and SDH, respectively).

RESULTS: Taking advantage of newly and previously developed qPCR assays, the frequency of key alterations associated with DMI (CYP51-S524T) and SDHI (SDHC-T79N/I, C-N86S and C-H152R) resistance was assessed in *Z. tritici*-infected wheat leaf samples collected from commercial crops (n = 140) across 14 European countries prior to fungicide application in the spring of 2019. This revealed the presence of a West to East gradient in the frequencies of the most common key alterations conferring azole (S524T) and SDHI resistance (T79N and N86S), with the highest frequencies measured in Ireland and Great Britain. These observations were corroborated by sequencing (*CYP51* and *SDH* subunits) and sensitivity phenotyping (prothioconazole-desthio and fluxapyroxad) of *Z. tritici* isolates collected from a selection of field samples. Additional sampling made at the end of the 2019 season confirmed the continued increase in frequency of the targeted alterations. Investigations on historical leaf DNA samples originating from different European countries revealed that the frequency of all key alterations (except C-T79I) has been gradually increasing over the past decade.

CONCLUSION: Whilst these alterations are quickly becoming dominant in Ireland and Great Britain, scope still exists to delay their selection throughout the wider European population, emphasizing the need for the implementation of fungicide antiresistance measures.

Supporting information may be found in the online version of this article.

Keywords: *Mycosphaerella graminicola*; sensitivity; disease control; septoria tritici blotch; triazole; wheat

1 INTRODUCTION

Septoria tritici blotch (STB) is a wheat disease caused by the ascomycete pathogen *Zymoseptoria tritici*. The disease occurs annually throughout Europe, with severity of infections reflective of meteorological conditions.[1–3] The use of fungicides is currently relied on to control the disease and secure both yield and grain quality. For the past two decades, the range of fungicide modes of action (MoA) effective against *Z. tritici* has been limited to the demethylation inhibitors (DMIs; mostly azoles) targeting ergosterol synthesis, the succinate dehydrogenase inhibitors (SDHIs) targeting fungal respiration, and a small number of multisite

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fungicides (e.g. chlorothalonil). Prior to this, additional MoAs were equally effective against *Z. tritici*, including the quinone outside inhibitors (QoIs) and methyl benzimidazole carbamates (MBCs). Unfortunately, alterations occurring in the genes coding for the targeted proteins of both MoAs, β-tubulin (E198A) and cytochrome *b* (G143A) for MBCs and QoIs, respectively, occurred in European *Z. tritici* populations and were quickly selected.[4,5] This resulted in a dramatic reduction in efficacy for both MoAs, rendering these fungicides obsolete for STB control throughout most of Europe.[5–7] Nevertheless, some control against STB can still be achieved using QoIs in east Europe.[4,8] A similar resistance development and selection situation, albeit at slower pace, is currently occurring toward the DMIs and SDHIs.[9,10]

DMIs were first developed and applied to wheat crops about 40 years ago for the control of diseases such as mildew and eyespot, caused by *Blumeria graminis* f. sp. *tritici* and *Oculimacula yallundae/acuformis*, respectively, and have also been the backbone of STB control for more than two decades.[9] This long history of use on wheat crops has led to a continual exposure of *Z. tritici* to this MoA, and as a result the pathogen has over time developed varying degrees of resistance to these fungicides. In most cases, resistance has resulted from changes in the target site, 14α-demethylase (CYP51), with the first amino acid alterations occurring in the early 1990s.[11] Since then, new alterations and subsequent combinations of these have evolved steadily over the following years, leading to a progressive decrease in DMI efficacies in the field.[9,12–19] The S524T alteration is one of the more recently reported and is present in most recently evolved highly resistant haplotypes.[15,17] This alteration has been suggested to have contributed to the decrease in field efficacy of prothioconazole and epoxiconazole.[20] The SDHI fungicides were also developed over 50 years ago (e.g. carboxin). However, it is only in the past decade following the commercialization of a new generation of SDHIs (e.g. bixafen, fluxapyroxad and benzovindiflupyr) that they have become an integral component of STB control in Europe.[21] In recent years, multiple alterations have been found in subunits B, C, and D of succinate dehydrogenase (SDH), which form the SDHI binding site. So far, most alterations affecting SDHI binding and subsequent SDHI resistance have been found in SDHC. Amongst these, C-T79N and C-N86S confer a moderate resistance phenotype, while C-H152R gives complete resistance to SDHIs. However, some studies suggest that C-H152R comes with a fitness cost to the pathogen.[22–25] Non-target site resistance mechanisms, such as the overexpression of CYP51 affecting DMI sensitivity, as well as overexpression of the efflux pump MFS1, belonging to the major facilitator superfamily (MFS) family of transporters and associated with a multidrug-resistant phenotype (including QoIs, SDHIs and azoles), have also emerged *in Z. tritici* populations.[26,27]

The distribution and frequencies of fungicide resistance in European *Z. tritici* populations appear be non-uniform across the continent, with regional differences even reported within countries.[13,15,17,19,24] From past and most recent European-wide sensitivity monitorings, the British and Irish populations seem to be most affected, with a gradient of sensitivity across European populations, increasing from west to east.[15,16,19,24] This pattern is likely reflective of the differences in disease pressures due to geographic location and climate, and the availability of specific fungicides and local agronomy.[28] However, these monitoring studies are often conducted using a limited number of samples from either treated or untreated trial plots across the region. Hence, although they may indicate regional sensitivities, they do not provide the detailed overview required.

Such detailed monitoring studies are important for a number of reasons. Fungicide resistance is highly dynamic whereby genetic alterations leading to resistance appear randomly in both space and time.[6,29,30] Due to the nature of the lifecycle of *Z. tritici* and development of STB, if these events coincide with periods of fungicide exposure, those strains that have acquired resistance are likely to have a strong fitness advantage over the wider sensitive population. Under such conditions, the resistant allele can rapidly become dominant in local populations and further mutate. Alongside this, as *Z. tritici* undergoes regular sexual reproduction throughout the growing season resulting in the production of wind-dispersed ascospores, such resistance has the potential to readily spread and be selected for on a regional scale and potentially over wider national scales. Brunner *et al.* (2008) hypothesized that this has been how the early CYP51 alterations spread throughout Europe. Indeed, resistant alleles have been detected in the airborne inoculum of *Z. tritici* in the case of QoI,[31] resistance conferred by G143A, and more recently for the CYP51 alteration S524T.[32] Therefore, it is imperative to monitor the sensitivity of *Z. tritici* populations at local, regional, national, and international scales.

Various research institutes and fungicide manufacturers perform annual resistance monitoring of national *Z. tritici* populations throughout Europe.[33] Undoubtedly, this provides precious information at a national level. As continuity in terms of sampling and sensitivity monitoring methods is not always the case, comparisons of sensitivity across the continent can be difficult. Publicly available data are consequently scarce and difficult to compare among European countries. As part of the EURO-RES project (EUROWHEAT Fungicide Resistance Network; https://agro.au.dk/forskning/internationale-platforme/eurowheat/) a standardized leaf sampling protocol was established and applied to winter wheat fields across Europe in spring 2019 before fungicide applications. Using a step-down process, first confirming the dominant target site alterations of relevance for both azole and SDHI resistance across the continent and subsequently using qPCR assays, both previously reported[32] and newly developed, the prevalence of these alterations throughout the region was determined. In addition, temporal changes in the frequencies of the tested alterations in countries contrasting in sensitivity were examined. The consequences of these findings in relation to the

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Table 1. Summary of the occurrence of targeted resistance alterations in *Zymoseptoria tritici* measured by qPCR in fields sampled across Europe in the Spring of 2019

	Alteration %																							
	Cyp51 - S524T				SdhC - T79N				SdhC - T79I				SdhC - N86S				SdhC - H152R				All SdhC[¶]			
Country (# of fields)	Mean[*]	Detection[†]	Max[‡]	Min[§]	Mean	Detection	Max	Min	Mean	Detection	Max	Min	Mean	Detection	Max	Min	Mean	Detection	Max	Min	Mean	Detection	Max	Min
Belgium (16)	16,9^{CD}	100	48,1	6,1	2,1^{C}	100	15	<0,1	0^{B}	0	0	0	2,7^{B}	100	12,3	<0,1	<0,1^{AB}	25	0,2	0	4,8^{CD}	100	26,3	0
Denmark (17)	8,3^{DE}	100	17,7	2,5	0,5^{C}	70,6	2,7	0	0^{B}	0	0	0	0,3^{B}	58,8	2,7	0	<0,1^{B}	5,9	<0,1	0	0,8^{CD}	82,3	2,9	0
Estonia (4)	1,1^{E}	75	1,7	0	0^{C}	0	0	0	0^{B}	0	0	0	0^{B}	0	0	0	0^{B}	0	0	0	0^{D}	0	0	0
Finland (2)	0,4^{E}	100	0,7	<0,1	0,1^{C}	50	0,1	0	0^{B}	0	0	0	0^{B}	0	0	0	0^{B}	0	0	0	0,1^{D}	50	0,1	0
France (18)	10,9^{DE}	100	21,9	3,3	0,8^{C}	44,4	9,6	0	0^{B}	0	0	0	<0,1^{B}	16,7	0,2	0	0^{B}	0	0	0	0,8^{D}	44,4	9,6	0
Germany (21)	21,5^{C}	100	35,5	10,4	4,5^{C}	100	12,7	0,1	<0,1^{B}	9,5	0,4	0	1,3^{B}	76,2	12,6	0	0,4^{AB}	23,8	4,6	0	6,3^{C}	100	25,3	0,1
Great Britain (16)	51,1^{B}	100	63,8	33,1	21,3^{B}	87,5	45,9	0	0^{B}	0	0	0	23,3^{A}	100	44,3	1,2	0,2^{A}	81,3	0,9	0	44,8^{B}	100	67	4,1
Ireland (14)[‖]	71,2^{A}	100	87,7	53,1	47,3^{A}	92,9	100	0	0,8^{A}	64,3	3,8	0	24,7^{A}	100	60,5	0,7	0,1^{AB}	64,3	0,5	0	72,9^{A}	100	107,5	35,5
Latvia (3)	4,8^{E}	66,7	12,6	0	0,6^{C}	33,3	1,7	0	0^{B}	0	0	0	0^{B}	0	0	0	0^{B}	0	0	0	0,6^{CD}	100	1,7	0
Luxembourg (7)	8,6^{DE}	100	15,7	3,9	2^{C}	100	3,9	0,2	0^{B}	0	0	0	0,4^{B}	85,7	1,1	0	0^{B}	0	0	0	2,4^{CD}	33,3	4	0,2
Norway (4)	13,6^{CDE}	100	38	1,2	0,8^{C}	75	2,5	0	0^{B}	0	0	0	<0,1^{B}	25	<0,1	0	0^{B}	0	0	0	0,8^{CD}	75	2,5	0
Poland (4)	3,7^{E}	100	9,9	<0,1	0^{C}	0	0	0	0^{B}	0	0	0	0^{B}	0	0	0	0^{B}	0	0	0	0^{D}	0	0	0
Sweden (13)	7,5^{E}	100	41,4	0,6	0,3^{C}	69,2	0,8	0	0^{B}	0	0	0	0,1^{B}	61,5	1,2	0	0^{B}	0	0	0	0,4^{CD}	69,2	1,5	0
Switzerland (1)	8,3^{DE}	100	8,3	8,3	<0,1^{C}	100	<0,1	<0,1	0^{B}	0	0	0	0^{B}	0	0	0	0^{B}	0	0	0	<0,1^{D}	100	0	0

[*] Average percentage of alteration among the field samples in each country. Different uppercase letters between countries correspond to significantly different means determined by Tukey's test.
[†] Percentage of fields in which the alteration was detected.
[‡] Maximal alteration percentage measured in a field.
[§] Minimal alteration percentage measured in a field.
[¶] Cumulated percentage of alterations in the SdhC subunit.
[‖] Including both Ireland and Northern Ireland.

Table 2. Oligonucleotides designed for the specific quantification of T79N/I and N86S alleles

Oligo name	Sequence*	5′/3′ modifications	Tm (°C)†
	Probes		
SdhC-79T-probe	GTACCAG**G**TTATTTG	FAM/BHQ-1	55,9
SdhC-79N-probe	GGTACCAG**T**TTATTTG	HEX/BHQ-1	55,1
SdhC-79I-probe	GGTACCAG**A**TTATTTG	TEX/BHQ-2	55,3
SdhC-86N-probe	CTCA**A**CCGCGT	FAM/BHQ-1	54,8
SdhC-86S-probe	CTCA**G**CCGCGT	HEX/BHQ-1	56,9
Primers			
SdhC-79-86-F	CACCTCGCAATCTACAAAC	—	51,2
SdhC-79-86-R	TCAACGAATGAAACGTCAC	—	50,7

*The positions of LNA bases in the oligonucleotide sequence are indicated by underlined bases. The nucleotides related to the amino acid change leading to a resistant phenotype are indicated in bold.
† Melting temperatures are only indicative and were obtained using the IDT OligoAnalyzer webtool with default parameters and no 5′/3′ modifications.

continued use and efficacy of azoles and SDHIs across the continent are further discussed.

2 MATERIALS AND METHODS

2.1 Field sampling

During the spring of 2019, 140 wheat fields were sampled for *Z. tritici* throughout 14 European countries (Tables 1 and S1). The fields were sampled before any fungicide treatment was applied and generally before wheat growth stage (GS) 39. In each field, a single leaf with STB symptoms (necrotic lesions and visible pycnidia) was sampled every 10–20 m along a W-shaped path across the fields. Leaf samples were subsequently air-dried and stored in paper envelopes at room temperature before further processing. An additional round of sampling was performed at GS 75–85 (post-fungicide application), with fields sampled in spring re-sampled in Belgium ($n = 8$) and Ireland ($n = 8$). Historical wheat leaf samples with STB post-fungicide treatment from 2011 to 2018 were recovered in Belgium, Ireland, and Denmark (Table S1) from national collections in the respective countries to observe the progression of the targeted alterations overtime.

2.2 *Z. tritici* isolation, sensitivity testing, and target site sequencing

To assess the *in vitro* sensitivity of *Z. tritici* from across the region, isolations from STB lesions originating from the 2019 pre-fungicide sampling of fields in Belgium ($n = 2$), Denmark ($n = 3$), Germany ($n = 5$), Ireland ($n = 3$), and Sweden ($n = 3$) were conducted as described in Dooley *et al.* (2016b). A total of 20 strains per field were generally recovered and conserved at −80 °C in 30% glycerol. The sensitivity of the strains to the SDHI fluxapyroxad (FLX) and the azole prothioconazole-desthio (PDZ) was determined using a microtiter plate assay using a method,[34] with the modification that the PDZ concentration range was set to 0, 0.01, 0.123, 0.37, 1.1, 3.3, and 10 mg L^{-1}. Moreover, minimum and maximum concentrations of both actives were modified for isolates where initial concentrations failed to provide sufficient growth or inhibition to allow for an EC50 calculation (FLX highest concentration increased to 40 mg L^{-1} and PDZ lowest concentration reduced to 0.001 mg L^{-1}). Technical grade fungicides were purchased from Sigma-Aldrich/Merck.

The complete *CYP51*, *SDHB*, *C*, and *D* subunits were amplified and sequenced in strains isolated from a single field in each of the above countries. Amplification of *CYP51* followed the procedure described by Huf *et al.* (2018). *SDH* subunits were aplified as described in Dooley *et al.* (2016b) for *SDHB*, Rehfus *et al.* (2018) for *SDHD*, and a combination of both methods for *SDHC*.

2.3 DNA extraction from leaf samples

A single STB lesion was identified on each leaf and excised using a 5 mm puncher. From each field, a maximum of four subsamples, each containing 10 excised leaf cores, was obtained. Each subsample (10 excised cores) was placed in a well of a 96-deepwell plate containing a 5 mm glass bead, freeze dried and ground into a fine powder using Retsch MM400 (Retsch). DNA was subsequently extracted from all subsamples on a KingFisher DNA extraction system using MagMax DNA extraction kit in accordance with the manufacturer's instructions (Thermo Fisher Scientific, Dublin, Ireland). Purified DNA was eluted in a final volume of 100 µL.

2.4 Determination of allele frequency in leaf samples

The frequency of the CYP51 alteration S524T and SDHC alterations T79N/I, N86S, and H152R was determined using three allele-specific qPCR assays. The determination of S524T and H152R frequency was performed simultaneously using a previously designed assay.[32] Two new assays were developed for the quantification of C-T79N/I (simultaneously) and C-N86S, following the criteria previously described.[32] Each of those two assays used the same set of primers, amplifying a 235 bp fragment, with probes specific to the targeted SNPs (Table 2). Primers and LNA-based double-dye probes were developed using reference sequences corresponding to each allele (T79, N79, I79, N86, and S86). Because of the requirement of a small probe size for reliable SNP detection and low GC content in the target sequences, up to six LNA bases were included in the design of each newly designed probe to reach a sufficient melting temperature (Tm). The cycling protocol consisted of an activation step at 95 °C for 3 min, followed by 40 cycles of denaturation (95 °C for 3 s) and annealing/extension (60 °C for 45 s), with the intensity of fluorescence captured at the end of every cycle. All reactions were performed on a CFX96 thermocycler (BioRad) using Takyon No Rox Probe MasterMix dTTP Blue (Eurogentec, Belgium). Each reaction was run in 20 µL of final volume and included 2 µL of template DNA and primers and probes at 100 nM and 250 nM,

Table 3. Sensitivity of *Zymoseptoria tritici* populations collected from fields in Belgium, Denmark, Germany, Ireland, and Sweden prior to fungicide treatment in spring 2019 to the azole prothioconazole (PDZ) and the SDHI fluxapyroxad (FLX), and frequencies of CYP51-S524T and SDHC-T79N/I, N86S, and H152R in bulk DNA from leaf samples from each field

				Sensitivity (EC_{50} mg L^{-1})		Frequencies of CYP51 and SDHC alterations in each field[a]				
Field I.D.	Country	Location	No. of isolates	PDZ	FLX	% S524T	% T79N	% T79I	% N86S	% H152R
1B	Belgium	Ath	5	0.22 (0.06–0.54)	0.44 (0.07–1.71)	9.27	1.03	0	0.74	0.03
2B	Belgium	Lonzée	5	0.19 (0.07–0.32)	0.23 (0.10–0.50)	7.79	0.1	0	0.46	0.23
10D	Germany	Einbeck-Holtensen	22	0.18 (0.04–1.00)	0.54 (0.06–3.31)	14.44	9.23	0	0	0
1D	Germany	Wassertrudigen	22	0.21 (0.003–1.11)	0.48 (0.04–3.34)	17.85	0.031	0	0	0
5D	Germany	Kastorf	20	0.41 (0.01–1.90)	0.93 (0.07–4.19)	31.52	4.16	0	0.3	0
6D	Germany	Bunde	17	0.59 (0.07–4.82)	4.35 (0.07–40.0)	29.26	11.35	1.58	3.78	4.58
IR2[b]	Ireland	Moorepark	9	2.02 (0.57–4.49)	9.05 (0.99–17.32)	87.71	0	0	42.2	0
IR3[b]	Ireland	Moorepark	10	1.00 (0.05–3.22)	4.15 (0.10–10.0)	11.02	3	1.38	0.15	0
IR4	Ireland	Kildalton	8	0.45 (0.06–1.10)	2.49 (0.12–6.04)	62.41	35.33	1.76	10.15	0
IR5	Ireland	Oak Park	8	0.68 (0.06–2.23)	2.59 (1.15–5.96)	70.56	58.93	0	2.1	0
DK1	Denmark	Flakkebjerg	13	0.35 (0.005–2.93)	0.24 (0.06–0.53)	6.70	2.01	0	0.15	0
DK2	Denmark	Horsens	16	0.20 (0.01–1.22)	0.62 (0.10–5.79)	5.57	0	0	2.69	0
SWE1	Sweden	Kalmar	21	0.03 (0.01–0.11)	0.20 (0.03–0.87)	4.37	0	0	0	0
SWE2	Sweden	Mörbylånga	13	0.08 (0.03–0.28)	0.17 (0.05–0.52)	3.51	0.02	0	0	0
SWE3	Sweden	Lidköping	18	0.07 (0.02–0.48)	0.12 (0.03–0.36)	4.08	0	0	0	0

[a] frequency of resistance alterations measured by qPCR in bulk DNA samples
[b] Samples were collected in the same field on different wheat cultivars (IR2=Extase and IR3=Cellule). Results obtained on Cellule were only used for the purpose of testing the sensitivity of the qPCR assays compared to EC50 values of strains isolated in the same fields

respectively. For the newly developed assays, the cycle threshold (CT) values were determined above a baseline threshold of 50 and 150 relative fluorescence units (RFU) for positions 79 and 86, respectively. All PCR runs included serial dilutions of standards and each reaction was performed in duplicate. For each 'unknown' sample, the frequency of a mutation was estimated by computing the ratio between the quantity of mutant for this allele, given by the respective probe signal, and the total quantity of *Z. tritici* DNA, deduced from the signals from all probes in the assay.

2.5 Data analysis

Statistical differences in the sensitivity of the populations between countries were determined by ANOVA followed by a Tukey HSD test. The sensitivity of groups of strains harbouring particular mutations were statistically compared to the sensitivity of the WT strains using Student's *t*-test ($\alpha = 0.05$). The same test was used to compare the frequency of mutations between samples collected pre- or post-treatment in each country. Frequency and sensitivity data were always transformed (arcsine square-root transformation and $\log_{10}$ transformation, respectively) prior to statistical analysis. Correlation between sensitivity and mutation frequency was performed using linear regressions. Mapping of resistance frequency was produced using QGIS 3.0. For practical reasons, Ireland and Northern Ireland were considered as the same territory when data were summarized by country.

3 RESULTS

3.1 *In vitro* sensitivity of European field populations of *Z. tritici*

A total of 205 *Z. tritici* strains from five countries were successfully isolated and their sensitivity tested to the azole prothioconazole-desthio

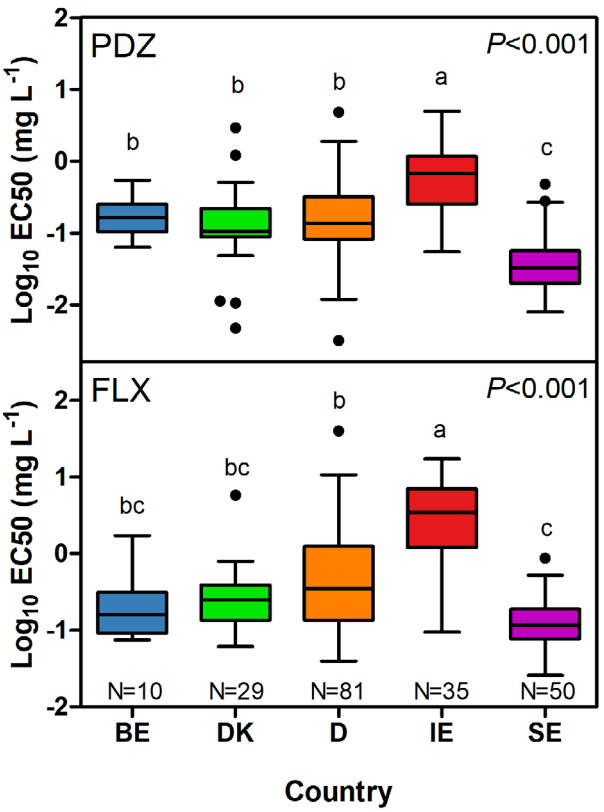

Figure 1. Differences in prothioconazole-desthio (PDZ) and fluxapyroxad (FLX) sensitivity between the five European *Zymoseptoria tritici* populations. Number of isolates: Belgium (BE) = 10; Denmark (DK) = 29; Germany (D) = 81; Ireland (IE) = 35; Sweden (SE) = 50.

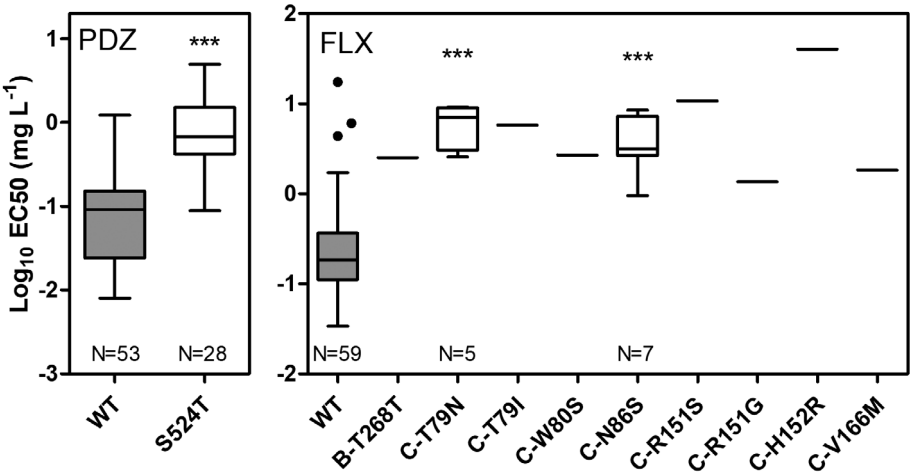

Figure 2. Comparison of EC_{50} values (mg L^{-1}) to prothioconazole-desthio (PDZ) and fluxapyroxad (FLX) of *Zymoseptoria tritici* isolates harboring different fungicide target protein alterations. For PDZ, wild-type (WT) includes all CYP51 haplotypes that do not harbor the S524T alterations whereas S524T includes all haplotypes with S524T. For FLX, WT includes haplotypes with no alterations in the SDHB, C and D as well as haplotypes containing only the N33T and N34T alteration in the SDHC. Floating lines represent the EC_{50} value obtained for single isolates.

and the SDHI fluxapyroxad. Total numbers of strains per field and country varied (Table 3). A large range in sensitivity was observed within the strain collection for both chemicals, with resistance factors (least sensitive divided by most sensitive) of 1565 and 1559 for PDZ and FLX, respectively. Significant differences in sensitivity to both chemicals were detected between the countries (Fig. 1). For both chemicals the Irish collection was the least sensitive and the Swedish the most sensitive. The Irish collection was significantly less sensitive to both chemicals compared to the other collections. No differences in sensitivity were observed between the Belgian, Danish, and German collections to PDZ or FLX, and all were significantly less sensitive to the PDZ compared to the Swedish collection, but amongst these collections only the German collection was significantly less sensitive to FLX when compared to the Swedish collection.

3.2 Impact of CYP51 alterations on azole sensitivity

The complete *CYP51* gene was successfully sequenced in 75 isolates originating from the fields 1B, 2B, 6D, IR2, IR4, DK2, and SWE1 (Table 3). Amongst these 21 different CYP51 amino acid haplotypes were identified, with the frequencies of each highly dependent on the country of origin (Fig. S1). The relative complexity of CYP51 haplotypes was different among the countries (haplotypes complexity IE > D > BE > DK > SE). Alterations at the key amino acid positions 136 and 381 were found in all collections. The S524T alteration was also present in all collections except the Swedish one. Furthermore, with the exception of a single isolate from Ireland, S524T was always found in combination with V136A/C and I381V. In the single Irish isolate, S524T was found in combination with V136A. Comparisons of the sensitivity of the strains with or without S524T (the 5′ end of CYP51 was successfully sequenced in an additional six isolates) clearly demonstrated that in contemporary European *Z. tritici* populations, S524T can be used as a marker to indicate PDZ sensitivity, with the mean sensitivity of strains with T524 10× lower to PDZ than those with S524 (Fig. 2; $P < 0.001$). This was reflected in the proportion of isolates with alterations in each field collection with frequencies found in the order of IE > D > BE > DK. Amongst the Irish and wider collection, isolates with the CYP51 haplotype H7 (L50S, D134G, V136A, A379G, I381V, Δ459/460, S524T), following the CYP51 nomenclature of Huf *et al.* (2018), were the least sensitive to PDZ. It should be noted that a small number of strains with S524T were sensitive to PDZ, including

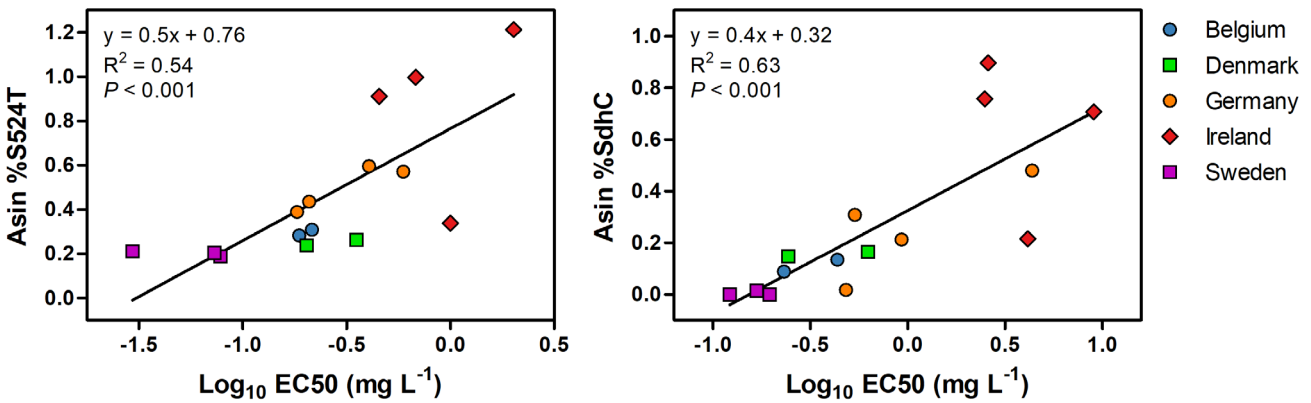

Figure 3. Correlation between the average sensitivity (log-transformed EC_{50} values) of *Zymoseptoria tritici* isolates to prothioconazole-desthio and fluxapyroxad and the frequency of key fungicide target protein alterations (arcsine square-root transformed frequency) measured by qPCR in the corresponding field. SDHC frequency in a field corresponds to the sum of T79N, T79I, N86S, and H152R alteration frequency. Data from field samples collected in the spring of 2019.

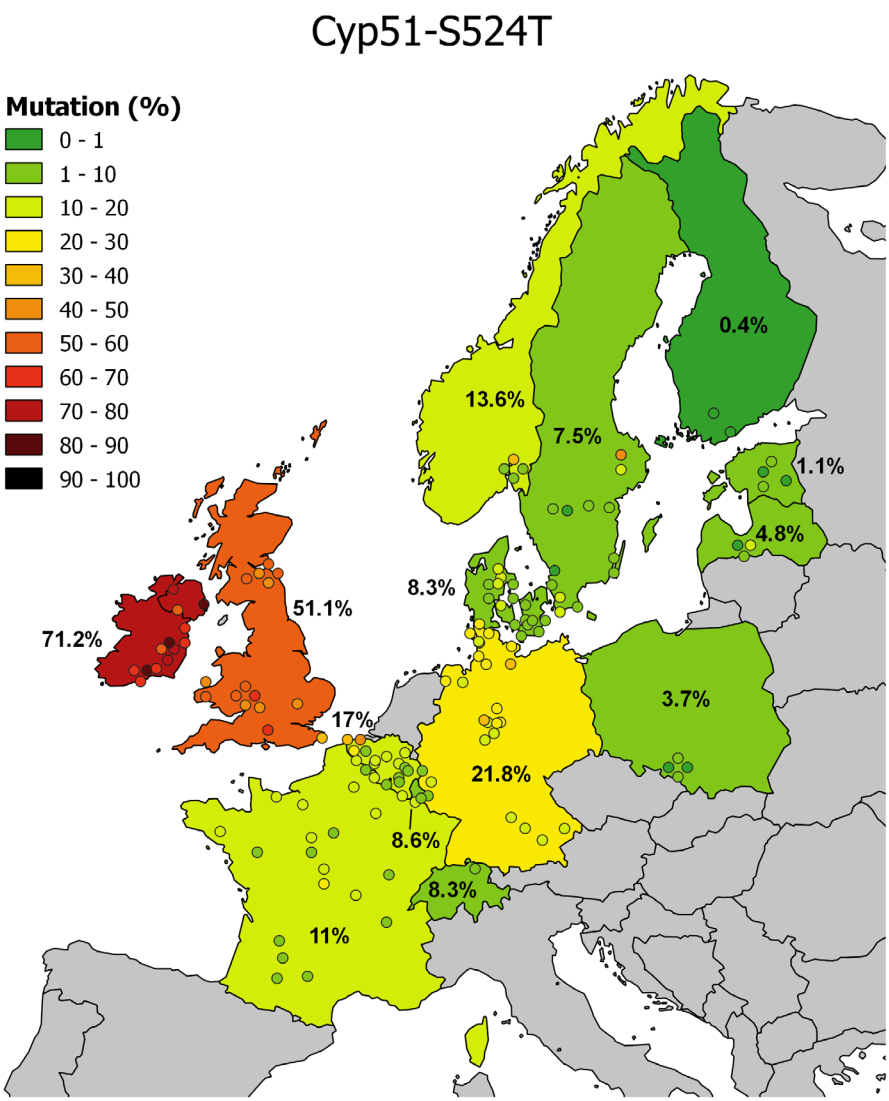

Figure 4. Spatial distribution of CYP51-S524T alteration in Europe measured in bulk leaf samples using qPCR. The color of each country represents the average percentage of alteration in the sampled field following the color legend (Ireland and Northern Ireland were considered as the same territory). The percentage of alteration in each field is also indicated by a colored dot. The countries in grey were not sampled.

the Irish isolate described above with haplotype F6 (L50S, V136A, S188N, Δ459/460, S524T) and isolates with haplotypes E7 (L50S, V136C, I381V, Y461H, S524T) and F4 (L50S, V136C, S188N, I381V, Y461H, S524T). Likewise, many isolates without S524T showed high EC_{50} values, including haplotypes C8 (L50S, I381V, Y461H) and G1 (L50S, S188N, A379G, I381V, Δ459/460, N513K).

3.3 Impact of SDH subunit alterations on SDHI sensitivity

A total of eight alterations at amino acid positions 79, 80, 86, 151, 152, and 166 in the SDHC subunit previously identified as contributing to SDHI resistance (FRAC, 2020) were identified in the collection, with a single strain with the alteration T268I in the SDHB subunit. No alterations associated with SDHI resistance were detected in the SDHD subunit. With the exception of a single isolate from Denmark, which had C-T79I, all isolates with alterations in their SDHB or SDHC associated with SDHI resistance were from either Germany or Ireland. Amongst the alterations in SDHC, both C-T79N ($n = 5$) and C-N86S ($n = 7$) were most frequent. A single strain with the alteration C-H152R was detected in the German collection. The presence of these alterations conferred the expected reduced sensitivity to FLX, with the most significant reduction found in the strain with C-H152R (Fig. 2). The EC_{50} values of strains harboring either C-T79N or C-N86S were significantly higher than WT strains ($P < 0.001$). On the other hand, no significant differences were detected when comparing the EC_{50} values of strains with C-T79N and C-N86S ($P = 0.22$). C-T79I appeared to have a similar impact on FLX sensitivity as C-T79N. Similar to CYP51-S524T, a small number of isolates without alterations in the different SDH subunits exhibited reduced sensitivity to FLX.

3.4 Frequency of CYP51-S524T and SDHC-T79N/I, C-N86S, and C-H152R alterations in the 2019 European *Z. tritici* populations

Both newly developed assays showed high specificity for the targeted alleles with no cross-reactivity (Table S2), allowing for accurate quantification of the specific alterations in DNA mixtures. The frequency of CYP51-S524T, SDHC-T79N/I, C-N86S, and C-H152R was determined in DNA from leaf samples originating from

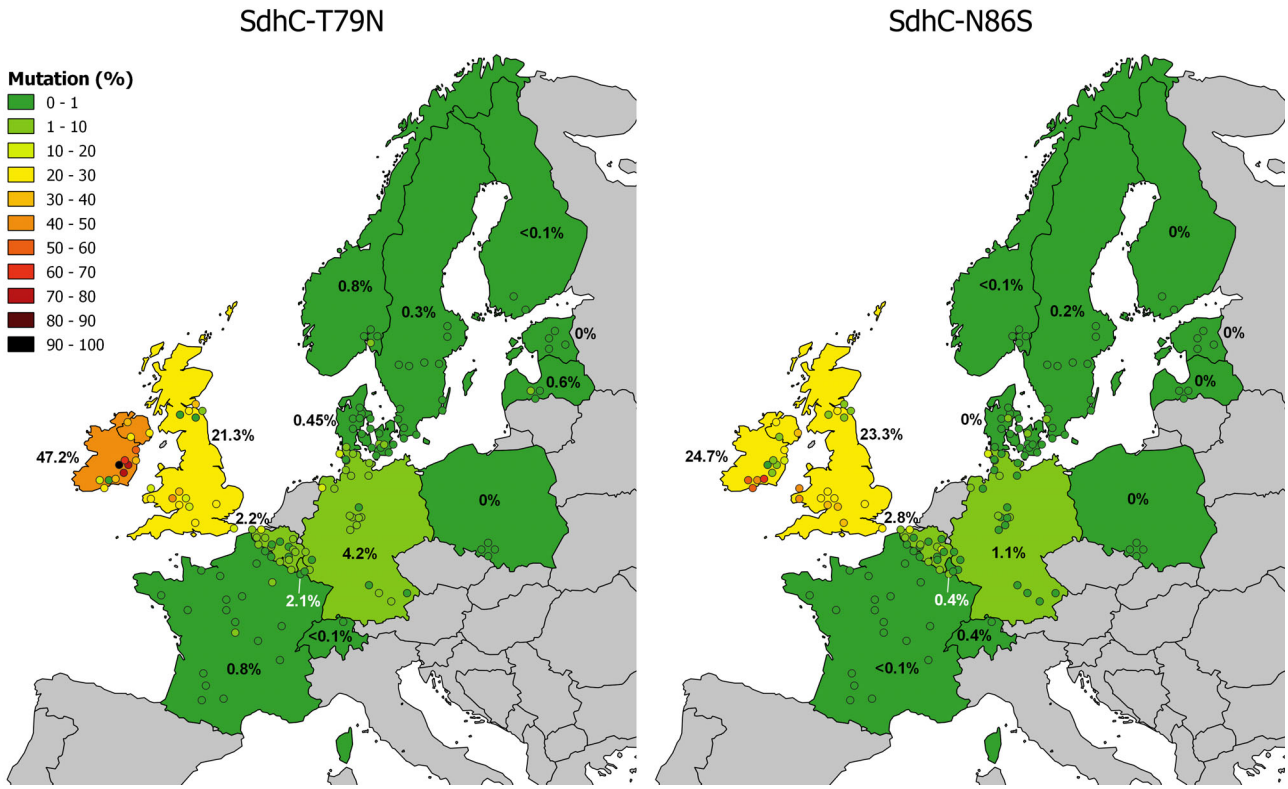

Figure 5. Spatial distribution of SDHC-T79N and N86S alterations in Europe measured in bulk leaf samples using qPCR. The color of each country represents the average percentage of alterations in the sampled field following the color legend (Ireland and Northern Ireland were considered as the same territory). The percentage of alteration in each field is also indicated by a colored dot. The countries in grey were not sampled.

140 wheat fields from multiple European countries (Table 1). In all fields where fungal isolations were made, the sensitivity of the *Z. tritici* isolates to fungicide molecules (PDZ and FLX) was correlated to the frequency of resistance alterations on the corresponding target gene (Fig. 3 and Table 3).

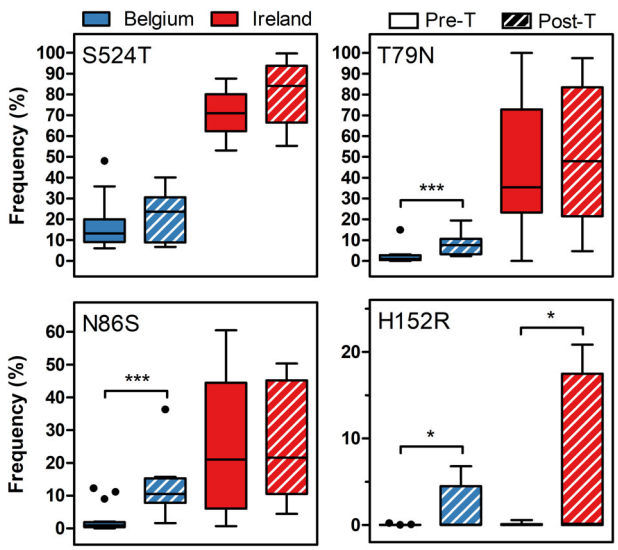

Figure 6. Average frequencies of resistance alteration measured by qPCR in samples collected before (Pre-T) and after (Post-T) fungicide application in Belgium and Ireland. Significant differences between pre- and post-treatment samples in each country are indicated with stars above the respective boxplots (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Box and whiskers are Tukey style.

Using these qPCR assays, strong significant differences in the occurrence of S524T, C-T79N, C-T79I, C-N86S, and C-H152R were observed among the different countries sampled ($P < 0.001$; Table 1). Ireland (including Northern Ireland) and Great Britain (GB) generally had the highest alteration frequencies, whereas the frequencies were generally much lower in Eastern countries.

Across all fields, S524T was the most frequent of the alterations assessed (22.3%), followed by C-T79N (8.4%), C-N86S (5.7%), C-H152R (0.09%), and C-T79I (0.08%). The S524T alteration was found in almost all fields (98.6%) (Table 1 and Fig. 4). The alteration was not detected in two of the 140 fields, one in Estonia and one in Latvia. A strong west–east gradient in frequencies of S524T was observed across Europe, with fields with the highest percentages of the S524T alteration found in Ireland (71.2%) and GB (51.1%). In continental Europe, the highest percentage of S524T was found in Germany at 21.5%. The lowest frequencies detected were in Finland, where less than 1% of S524T was found in the samples, although it must be noted that only two fields were sampled. Differences in frequencies of the alteration were also apparent within some countries (Fig. 4), with fields located in the same regions also having distinct frequencies of S524T. In France, Belgium, and Germany, a gradient from north to south was apparent, with more S524T present in the northern regions of each country.

The frequency of C-T79N and C-N86S followed a similar distribution as S524T. C-T79N could be detected in most countries (exceptions being Estonia and Poland), whilst the detection of C-N86S was a little less frequent (not detected in Estonia, Finland, Latvia, Poland, and Switzerland). For both mutations,

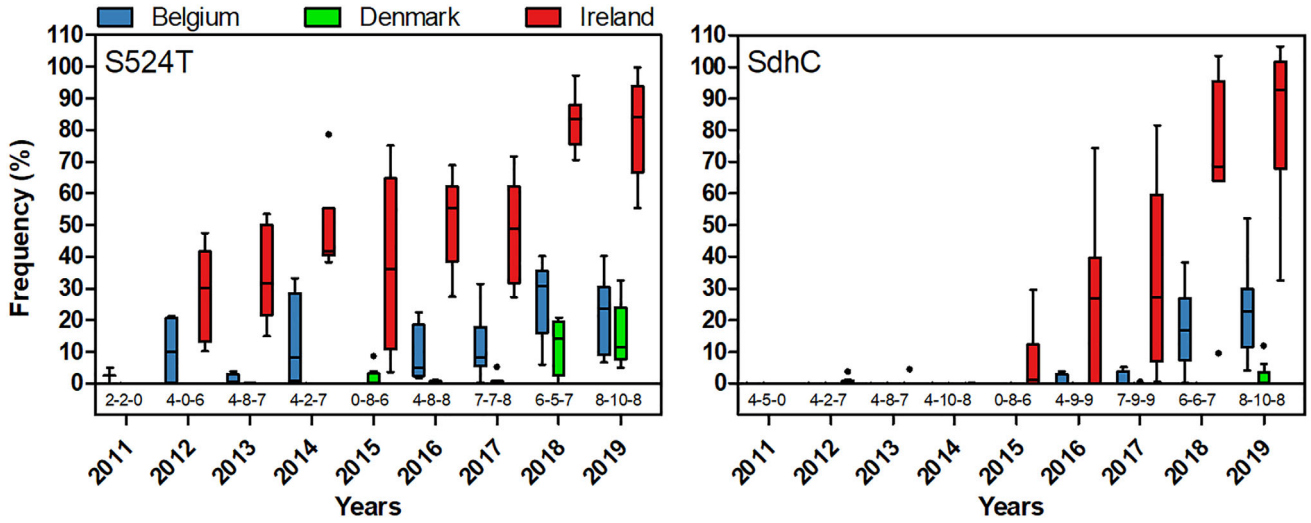

Figure 7. Temporal dynamics of the CYP51-S524T alteration and key SDHI alterations (cumulative) in Belgian, Danish, and Irish *Zymoseptoria tritici* populations originating from winter wheat fields sampled post fungicide treatment(s) in 2011–2019. The number of fields analyzed per country in each year is indicated under each box. Box and whiskers are Tukey style.

a large difference in the frequencies was found between Ireland and GB compared with the rest of Europe (Table 1 and Fig. 5). The frequency of C-N86S was relatively similar between GB and Ireland, with the highest frequency (60.5%) detected in an Irish field, whilst the mean frequency of C-T79N was higher in Ireland than in GB. In contrast, in continental Europe, most countries had very low percentages (<1%) of each alteration. Although the frequencies of C-T79N and C-N86S appeared to be a little higher in Belgium and Germany (and Luxembourg in the case of C-T79N) than in the rest of Europe, this difference was not significant (Table 1). At the country scale, some spatial differences are visible in GB, with slightly higher frequencies of C-T79N and N86S in the south than in the north. In Ireland, a similar spatial difference was observed, with fields showing either high C-T79N frequencies (east) or high C-N86S frequencies (south-west). The SDHC alterations T79I and H152R were only detected on rare occasions and at very low percentages (Table 1). C-T79I was only detected in Ireland (0.8%) and Germany (<0.1%), and also identified in a strain isolated from a field in Denmark. The highest frequency of this alteration measured in an individual field was 3.8% in an Irish field. C-H152R was detected in Germany (0.4%), GB (0.2%), Ireland (0.1%), Belgium (<0.1%), and Denmark (<0.1%). The highest frequency was detected in an individual field with 4.6% in a German field. Nevertheless, even though the overall frequency of C-H152R was low, it was detectable in the majority of fields in GB (81.3% of fields) and Ireland (64.3%). In the other countries where C-H152R was detected, overall frequencies of detection were considerably lower (25% in Belgium, 23.8% in Germany, and 5.9% in Denmark).

Cumulatively the frequency of alterations associated with SDHI resistance (C-T79N/I, N86S, and H152R) was highest in Ireland (mean frequency of 72.9%, with a maximal >107.5% recorded in a single field), which was significantly higher than in GB (mean frequency of 44.8%), which itself was significantly higher than the next closest country, Germany (mean frequency of 6.3%). The cumulative frequencies of the different alterations in the rest of the countries sampled were all below 5% (see Table 1 for further information).

3.5 Selection for resistance between pre-and post-fungicide applications in 2019

Overall, the percentage of each alteration was higher post fungicide treatment in both Belgium and Ireland, although only some of these differences were statistically significant (Fig. 6). Similar to the pre-treated samples, S524T was detected in all fields, with the highest frequencies again found in Irish fields (mean frequency 80.7%). In Belgium, the frequencies C-T79N and C-N86S were significantly higher post treatment. In Ireland, the frequencies of both alterations were not significantly different, however a closer examination of the frequencies of each mutations in individual fields at both sampling points shows the high variability between both alterations as highlighted earlier (Fig. S2). In Sweden, C-T79N was detected in the spring samples but not in the post-treatment samples. Similar to the Danish samples, different fields were sampled at each time point in Sweden, and this change in frequency may reflect these differences. C-H152R was detected in Ireland and Belgium in both pre-and post-treatment samples, with the frequency of the alterations significantly higher in both countries post treatment (Fig. 6). The alteration was not detected in all fields in both countries, but in Ireland, it was detected at up to 21% in one field and 6.8% in a field in Belgium (Fig. S2). The C-T79I alteration was only rarely detected (data not shown).

3.6 Temporal changes in frequency of CYP51-S524T, SDHC-T79N/I, N86S, and H152R in Belgium, Denmark, and Ireland

Using representative post-treatment commercial field samples, the temporal dynamics of the different alterations in Belgium, Denmark, and Ireland were examined from 2011 onwards. As previously highlighted, S524T, C-T79N, and C-N86S were detected in each of the three countries post treatment in 2019, whilst C-H152R and C-T79I were only detected in Belgium and Ireland. Differences were observed between the countries in terms of year when the alterations were first detected and subsequent selection (Figs 7 and S3). Common amongst the three countries was

a substantial increase in the frequencies of S524T and SDHC alterations between the years 2017 and 2018. The same trend is observable individually for each alteration in the SDHC, except for T79I (Fig. S3).

S524T was first detected in Belgium in a single field in 2011 (Fig. 7). Following this, its frequency in individual fields fluctuated, ranging from 0% to 40.2%, with overall mean frequencies of 22% and 24.1% in 2018 and 2019, respectively, compared to 11% in 2017. In Denmark, S524T was first detected in 2013. However, its overall frequency remained low until 2018, when detected at a mean frequency of 11.7%, ranging from 0% to 20.8% depending on the field sampled. In Ireland, S524T was already present in all fields sampled in 2012, the first season examined, at a mean frequency of 28.7%. This steadily increased to 48.3% in 2017, after which it increased to 83.3% in 2018 and remained at this high level in 2019.

Cumulatively the increase in the frequencies of SDHC alterations impacting SDHI activity has differed depending on the country (Fig. 7). SDHC alterations were first detected in Ireland in 2012 (first with T79I), but their frequency only started to increase from 2015. Their frequency thereafter increased every year, reaching an average of 83.2% (ranging from 32.5% to 106.5%) at the end of the 2019 season. The greatest increase in frequency of SDHC alterations was from 32.2% to 70.5% between 2017 and 2018. In Belgium, the first SDHC (both C-T79N and C-N86S) alterations were detected in 2012. As for Ireland, a substantial spread and accumulation of SDHC alterations was measured between 2017 and 2018, going from being present in less than 30% of the fields (mean = 1.3%) to 100% (mean = 17.5%). At the end of the 2019 season, the average SDHC frequency in Belgian wheat fields was 23.1% (4–52.1%). SDHC alterations were first detected in Denmark in 2017 (C-T79N) in a single field (out of nine). Interestingly, they were not detected in 2018, but in 2019 they were detected in 50% of the fields sampled with an overall average of 2.2% (0–11.9%).

Amongst the four SDHC alterations (Fig. S3), C-T79I was the first detected, with the alteration present in two Irish fields in 2012 and a single field in 2013. Its detection has been sporadic since with frequencies ranging from <1% to 26% (Fig. S3). The detection of C-H152R has also been sporadic, with its first detection in two Irish fields in 2016 at <0.1% frequency. Its detection has since then increased, with the alteration detected in four of the nine fields sampled in Belgium in 2019 and six of the eight Irish fields. C-H152R was not detected in any of the Danish samples examined.

C-T79N was first detected in a single field in Belgium in 2016 and again in a single field in 2017. In 2018 and 2019 it was detected in all fields, ranging in frequency from <0.2%to 19.4%. In Denmark, it was first detected in a single field in 2017 at 0.7%. In 2019, it was detected in five of the nine Danish fields sampled, ranging from 0.2% to 11.9%. In Ireland, C-T79N was first detected in a single field at 0.1% in 2014. In 2015 and 2016, it was present in four of the eight fields sampled, and by 2017 onwards it was detected in all fields, ranging in frequency from 1.4% to 97.5%.

C-N86S was first detected in Belgium in a single field in 2016 at <0.1%. Comparable with C-T79N, it has since increased in frequency and was detected in all fields by 2019, ranging in frequency from 1.6% to 36.3%. It was only detected in Denmark in 2019 and in three of the 10 fields sampled, ranging in frequency from 0.1% to 1.2%. In Ireland, C-N86S was first detected in 2014 (in a different field from that where C-T79N was detected) at 0.1%. Although its increase has not been as dramatic as C-T79N by 2018, it was present in all fields sampled, ranging in frequency from 0.3% to 60.9%.

4 DISCUSSION

Amongst the 205 isolates tested that come from five counties (BE, DK, D, IE, and SE), an extremely broad range of sensitivities was detected, with a resistance factor (most resistant divided by most sensitive) for PDZ of 1559 and for FLX of 1565. Whilst such broad ranges did not exist in most fields, they demonstrate the potential for resistance selection that now exists across the wider region. As identified by Brunner *et al.* (2008), the spread of fungicide resistance is unlikely to be locally confined in Europe, and the potential for the more resistant strains to migrate into the more sensitive populations is a real possibility. Sequence analysis of the genes for the different target sites (*CYP51* and *SDHC*, *B*, and *D*) identified the presence of previously identified alterations in those isolates exhibiting the highest level of resistance.[17,22]

Resistance to azoles is now more than ever related to a complexity of CYP51 haplotypes, evident in the present study, where 21 different amino acid haplotypes were present in the 75 strains investigated (Fig. S1). As might be anticipated from the sensitivity data, the Irish collection contained the more complex or evolved haplotypes, with haplotype H6 (L50S, V136C, S188N, A379G, I381V, Δ459/460, S524T) being the most common, whilst in the Swedish collection the more naïve, less evolved haplotypes dominated, with haplotype C8 (L50S, I381V, Y461H) most often detected. Common amongst most of the strains exhibiting resistance or reduced sensitivity to PDZ was the presence of the CYP51 alteration S524T. Since this observation is also supported by previous work,[15,17] this alteration was used in the present study as an indicator of azole sensitivity across the wider European *Z. tritici* population. Nevertheless, it should be noted that S524T may not always be a clear indicator of azole resistance. The alteration has previously been identified in GB (2001–2003 isolates), Denmark (2007), and New Zealand (2010) in combination with Y137F, and this haplotype (B11) confers relatively high levels of insensitivity to triadimenol (old azole, not available anymore) but less so to PDZ in comparison with other more recently evolved haplotypes carrying S524T.[20] Indeed, the sensitivity conferred by the different CYP51 haplotypes is highly dependent on the specific azole assessed. As prothioconazole (the precursor of PDZ) is currently a key azole in European cereal production, the frequency of S524T is a good indicator for the prevalence of reduced sensitivity amongst *Z. tritici* populations to this azole. As newer azoles continue to be commercialized, such as mefentrifluconazole,[35,36] additional or novel alterations or combinations of alterations may become more important and warrant specific monitoring.

Mutagenesis-based risk assessment studies focusing on the potential for SDHI resistance development and selection highlighted a number of alterations in *Z. tritici* which could potentially impact the efficiency of various SDHI fungicides,[23,25,37] amongst which SDHC- T79I, N86S and H152R were identified. Various *in vitro* sensitivity assessments from field isolates, including those presented here, have confirmed the impact of both mutations on SDHI sensitivity.[22,24] The alteration C-T79N was not identified in the risk assessment studies but has since been reported in field populations with decreased SDHI sensitivity, as confirmed in the present study.[24] Similar to the diversity of CYP51 alterations and impacts on azole sensitivity, numerous other key SDH alterations, sometimes in combination, have been identified as contributing to SDHI resistance,[38] with B-T268I, C-W80S, R151S/G, and V166M also identified at low frequencies in the isolate collections examined as part of this study. The combination of reported prevalences and implications of the different alterations for STB

control led us to focus on C-T79N/I, C-N86S, and C-H152R. Multiple isolates of the collection were less sensitive to FLX without having any alteration in their SDH subunits. This might be the result of an enhanced activity of the MFS1 transporter, although the resistance factor provided by this mechanism against SDHI is rather low.[18] Again, similar to the azoles, the continued development and commercialization of novel SDHI fungicides may impact differently on *Z. tritici*, leading to the development of novel alterations or combinations of alterations, and the key alterations to be targeted may need to change in the future to reflect this.[38]

Taking advantage of the previously developed methodology to quantify CYP51-S524T and SDHC-H152R in mixed-DNA samples,[32] complementary qPCR assays were developed to quantify the frequencies of C-T79N/I and C-N86S. The three assays allowed for the accurate detection of the five resistance alterations in leaf DNA samples (contemporary and historical) originating from more than 300 European wheat fields. The use of this combined-assay method as proxy for resistance monitoring was validated through its high correlation with data from actual strain sensitivity to both PDZ and FLX obtained with the traditional *in vitro* assays.

A clear west–east gradient across Europe in the frequencies of these key alterations was evident amongst the collections established in spring 2019. This gradient reaffirms the contrasting differences in frequencies identified in field trials focusing on *Z. tritici* from across the region for the azoles[39] and for the SDHIs.[24] Unlike both these reports, the isolate collections presented here are in most instances representative of commercial crops before fungicide applications in 2019 and span a wider geographic range in the majority of the countries sampled. The frequencies of the different alterations presented also support the original findings of Brunner *et al.* (2008), who suggested that azole resistance, which in the late 1990s/early 2000s was limited to a small number of individual CYP51 alterations, originated in north-western Europe and spread eastward, most likely via ascospores. A similar gradient was also observed in European *Z. tritici* populations when looking at the G143A alterations responsible for QoI resistance,[4,5] although Torriani *et al.* (2009) showed that G143A emerged independently at least four times in European populations.

The CYP51 alteration S524T was detected in almost all fields sampled in spring 2019, indicating how widespread this alteration currently is in European *Z. tritici* populations. In most fields outside of Ireland and GB this alteration is still at relatively low frequencies. However, it was detected at moderate levels in a small number of fields across the region. In Ireland and GB the alteration was detected in all fields and at high levels (71.2% in Ireland and 51.1% in GB), reflective of the relatively poor levels of efficacy provided by azoles observed in both countries in recent seasons.[9,39] Understanding why the Irish and GB populations have become dominated by S524T will be important if strategies are to be devised to prevent/delay its further spread and accumulation across Europe. Key to achieving this will be the ability to reduce the selective advantage that resistance strains, such as those with S524T, have over sensitive strains in the presence of a fungicide and the potential period of time over which this can occur.[40] Limiting the application of azoles will probably achieve this, but as azoles are a key component of wider cereal disease control strategies, not just for STB, this is very unlikely to be easy to achieve. Within the review conducted by van den Bosch *et al.* (2014), reducing the amount of fungicides applied and hence the duration over which fungicide resistance can be selected was identified as means through which selection for potential resistance can be reduced. In high disease pressure environments, such as Western Europe, the application of fungicides for STB control are often based on the perceived risks of infection. As the accuracy of disease forecasting increases, the ability to better target applications to actual risk events will potentially allow for reductions in the number of treatments and doses of fungicides applied.

Alternatively, as described by van den Bosch *et al.* (2014), mixing fungicides with different modes of action will reduce the advantages resistant strains of *Z. tritici* may have over sensitive strains in the presence of the fungicide in question. Indeed, this has been a key component of STB control strategies for the past decade and will likely continue to be.[41] In the case of azole resistance, Dooley *et al.* (2016b) demonstrated that the SDHI fungicides buffered the selection for azole resistance when applied with an azole. The trials described by Dooley *et al.* (2016b) were conducted using natural *Z. tritici* populations that were sensitive to the SDHIs. Following the emergence of SDHI resistance in Ireland and GB, the levels of azole resistance protection provided by the SDHIs most likely decreased, and hence the continued increase of S524T in both countries as evident from the increased frequency of S524T in Irish fields between 2016–2019.

Complementary to this, as the efficacy of the azoles weakened due to increased frequencies of resistance towards them in *Z. tritici* populations, the protection they afforded to the SDHIs is likely to have weakened. This may have allowed for the rapid accumulation of the different alterations associated with SDHI resistance in both Ireland and GB. As observed in the historical post-treatment samples from Ireland, this selection has been rapid. The accumulated frequency of the key alterations screened here dominated Irish crops in spring 2019, having been at an extremely low level post-treatment in 2015 and low levels in 2016. In a small number of Irish fields in 2019 the cumulative frequency of alterations impacting SDHIs was >100%, suggesting that multiple key alterations affecting SDHI sensitivity are present in the same strain. Whilst this may be an artificat of the detection system, laboraory mutants with multiple SDH alterations have been detected[37] and recent findings reported by FRAC (2020) demonstrate that such strains also exist in the field. Moreover, the presence of double mutants could be undermined as the frequencies of many other mutations were not measured (e.g. C-W80S and C-R151S/T) It is equally interesting to note that even though C-T79I was detected in Ireland as early as in 2012, it has not been further selected for. This may, of course, be due to additional factors other than the sensitivity of partner fungicides used in the early season, such as fitness penalties associated with C-T79I.

Such potential fitness penalties have also been suggested to be associated with the SDHC alteration H152R, which is known to confer high levels of resistance to currently available SDHIs.[23] Across Europe in spring 2019, this alteration was only detected in a small number of crops, and even in these crops it was only detected at extremely low frequencies. SDHC alterations that confer moderate but still significant SDHI resistance (C-T79N and N86S) were, however, detected in the majority of countries and the majority of crops. Both these alterations are now at high levels in both Ireland and GB, and are impacting the efficacy conferred by the various SDHIs.[10] Similar to the situation with the azoles, strategies must be put in place elsewhere in Europe to ensure those low levels of SDHI resistance that already exist are not further selected. In Ireland and GB as these alterations now dominate, the applications of both the azoles and the SDHIs will be required to be accurately timed to ensure protective activity. However tempting it may seem to increase the doses of both groups of fungicides to achieve the desired control, this will likely

further exacerbate the selection for strains with high levels of DMI and SDHI resistance, like CYP51 haplotype H7 (L50S, D134G, V136A, A379G, I381V, Δ459/460, S524T) and SDHC-H152R, respectively.

In addition to the west–east gradient in the frequencies of the different alterations, differences were detected within different regions. This was most pronounced for the frequencies of C-T79N and C-N86S in Ireland, with crops in the south of the country having higher proportions of N86S whilst those in the east were dominated by C-T79N. As regional differences in fungicide usage do not exist in Ireland, these differences more likely reflect where these alterations first emerged and were consequently rapidly selected at a local scale, although a role of other epidemiological factors (such as temperature) on the fitness of SDH haplotypes cannot be ruled out. Similar regional differences were detected in GB, with higher frequencies of C-N86S detected in England compared to Scotland. Regional differences may also exist for specific CYP51 haplotypes. Differences in the prevalence of S524T were evident in France, Belgium, and Germany, with populations collected from the north of each country having higher proportions of the alteration. In France, these differences have been attributed to differences in fungicide usage across the country, reflecting the differences in disease pressures.[28]

Overall, the present study confirms alterations known to confer resistance to the azole and SDHI fungicides are widespread throughout modern European *Z. tritici* populations. Differences in frequencies exist, with the high levels of the key CYP51 and SDH alterations detected in both Ireland and GB likely to impact the efficacy of azole and SDHI fungicides in these countries. Elsewhere in Europe, increasing frequencies of S524T suggest the efficacy of the azoles may also be impacted, but as the frequencies of the key SDH alterations known to confer SDHI resistance continue to remain low, the efficacy of the SDHIs should still be unaffected. Further research is required to determine what the impact is of regional gene flow in the spread of both azole and SDHI resistance, or whether fungicide target protein alterations are emerging independently in the different regions. Either way, anti-resistance measures, including agronomic practices that reduce the need for intensive fungicide regimes, must continue to be promoted and practiced to prolong the effective life of current and future fungicides in order to sustain STB control.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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