Annual Project Report 2022

Project title	Monitoring and understanding fungicide resistance development in cereal pathogens to inform disease management strategies		
Project number	21120018a		
Start date	1 April 2019	**End date**	31 March 2022

Project aim and objectives

Linked to the AHDB winter wheat fungicide performance trials, this project will establish baseline sensitivities for new actives entering the market and monitors shifts in sensitivity (phenotype-to-genotype relationships) in UK *Zymoseptoria tritici* (Zt) populations to all key fungicides belonging to different mode of actions (MOAs).

In addition, DNA-based diagnostic assays that target new genotypes will be developed to measure the spread and further selection of resistance mechanisms in field populations.

Knowledge on the evolution and accumulation of fungicide insensitive genotypes within populations will inform fungicide choice, timing, dose, and MOA partnering aspects in commercial crops.

The methods developed are generic and can also be applied to other major fungal foliar cereal pathogens, such as *Ramularia collo-cygni*, *Pyrenophora teres* and *Rhynchosporium commune*.

The five project objectives are:

1. Measure annual early-season *in vitro* fungicide sensitivities of septoria populations for key fungicides (azoles, SDHIs and MOAs entering the market). Samples are taken at various UK locations at the start of the season and compared with available baseline sensitivities of populations sampled in previous seasons
2. Measure the effect of various spray programmes on fungicide sensitivity shifts in populations sampled from the fungicide performance trials by comparing the fungicide sensitivity profiles of populations sampled after fungicide applications with those sampled from untreated plots
3. Establish which resistance mechanisms operate in the most insensitive septoria field isolates
4. Develop DNA-based assays to quantify the frequency of important fungicide resistant alleles within field populations
5. Knowledge transfer of the fungicide sensitivity status of septoria and other key cereal pathogens to assist resistance management

Annual Project Report 2022

Key messages emerging from the project
• No significant further shifts in azole insensitivity (prothioconazole-desthio and mefentrifluconazole) but populations are still adapting (increase of more complex CYP51 variants with V136C and S524T, and isolates with enhanced efflux pump activity) • Mefentrifluconazole selection for CYP51 variants is different than that of prothioconazole-desthio. Further field studies are needed to explore this in practise (enhanced septoria control) • Further shifts in SDHI insensitivity (removal of sensitive strains) and slow accumulation of C-H152R strains in populations is ongoing • Fenpicoxamid (a QiI) baseline studies show strong inhibition of *Z. tritici* during *in vitro* growth at very low concentrations • Monitoring of baseline sensitivities to new actives for septoria leaf blotch control likely to enter the market is ongoing (e.g. pydiflumetofen, isoflucypram, fluindapyr and metyltetraprole) and will be used to detect fungicide sensitivity shifts as soon as products enter the market • Enhanced efflux pump activity in some *Z. tritici* strains has reduced the sensitivity to azole, QoI, QiI and SDHI fungicides during *in vitro* growth, further studies are needed to assess the impact during *in planta* growth in the glass house and in field trials

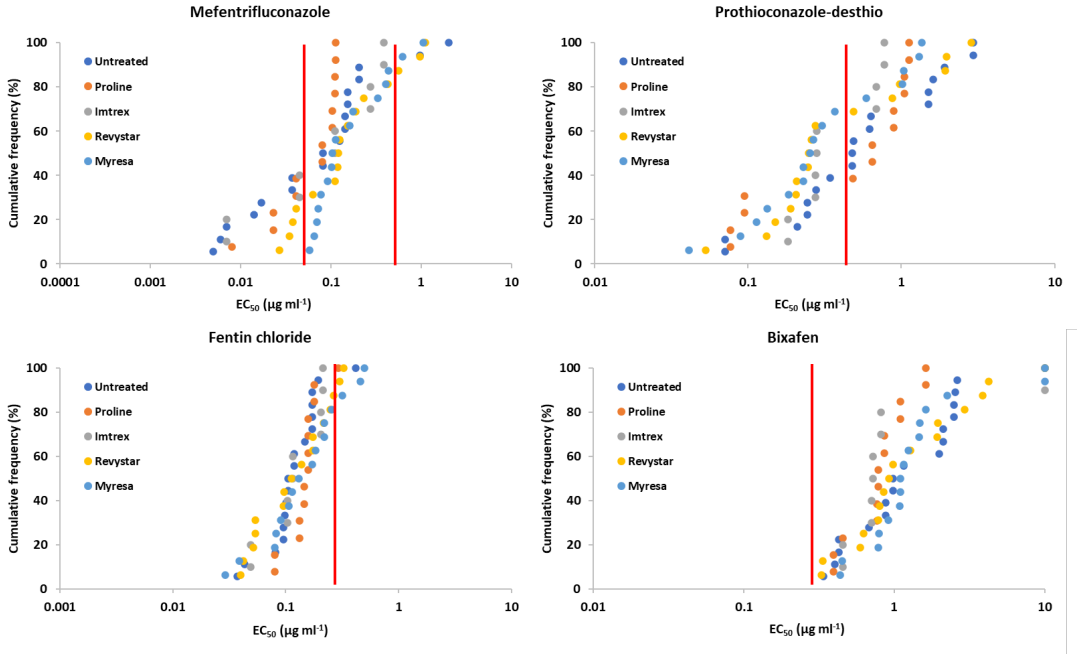
Annual Project Report 2022

Summary of results from the reporting year

Fungicide sensitivity shifts in the wheat fungicide performance trials during 2021

Populations of *Z. tritici* were sampled at Sutton Scotney (Hampshire), Cardigan (Wales), Perth (Dundee), Telford (Shropshire) and Caulshiel (East Lothian) and screened for fungicide sensitivity to study the effects of fungicide treatments based on single spray applications (T1, T1.5 or T2) of solo products (Proline 275 (a.i. prothioconazole)), Myresa (mefentrifluconazole) and Imtrex (fluxapyroxad)) or a mixture (Revystar XE (fluxapyroxad + mefentrifluconazole)) at recommended label rates. Figures 1 and 2 show the results for two locations, Sutton Scotney and Caulshiel, respectively.

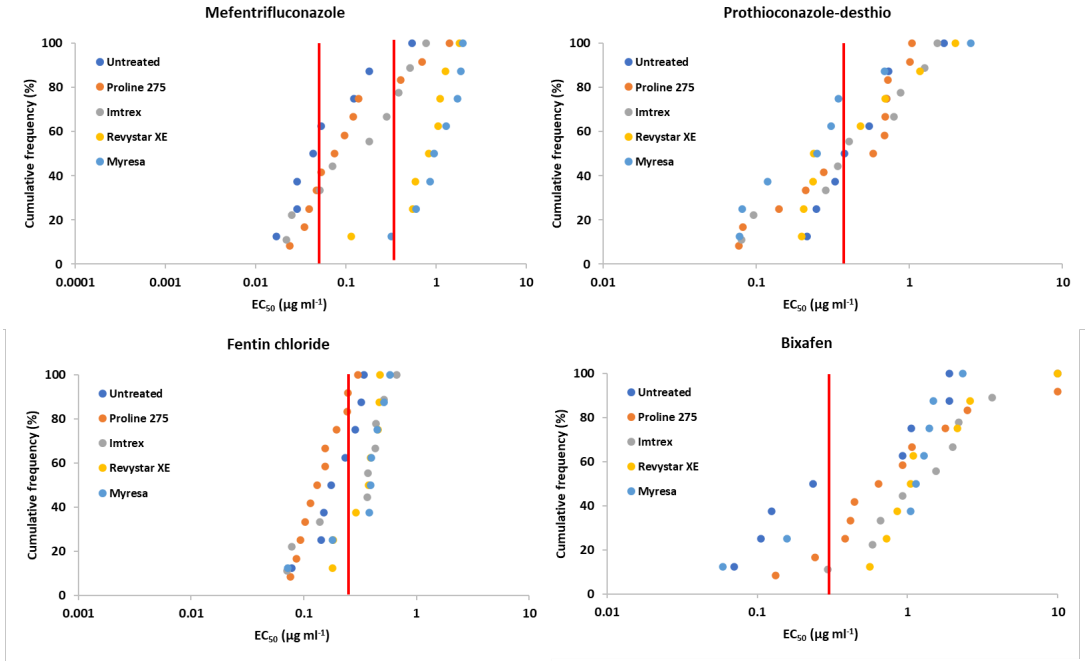
Figure 1. Fungicide sensitivity profiles of untreated and fungicide treated *Z. tritici* populations sampled at Sutton Scotney (Hampshire). Sampling was carried out 3-4 weeks after the T1 spray. Red vertical lines indicate sensitivity levels for mefentrifluconazole (0.05 and 0.4 ppm), prothioconazole-desthio (0.4 ppm), fentin chloride (0.25 ppm) and bixafen (0.3 ppm).



No obvious shifts in bixafen and fentin chloride sensitivity were observed at Sutton Scotney. As expected, less strains highly sensitive to mefentrifluconazole (EC_{50} <0.05 ppm) and more mefentrifluconazole insensitive strains (EC_{50} ≥0.4 ppm) were detected after treatments with Myresa and Revystar XE, products containing mefentrifluconazole as active. In comparison with untreated and the Proline 275 treated populations, more prothioconazole-desthio sensitive strains (EC_{50} <0.4 ppm)

were detected after treatments with Myresa and Revystar XE, indicating differences in selection for these two sterol 14α-demethylation inhibitors. Similar trends, but more pronounced were also observed for the trial carried out at Caulshiel (East-Lothian) (Figure 2). At this location, there was a clear shift towards metrifluconazole insensitivity (EC_{50} ≥0.4 ppm) after treatment with Myresa and Revystar XE. Both treatments also selected for fentin chloride insensitivity (isolates with EC_{50} ≥0.25 ppm), indicating isolates with increased efflux pump activity can tolerate higher levels of mefentrifluconazole during *in vitro* growth. In comparison with the untreated and Proline 275 treated populations, more prothioconazole-desthio sensitive strains (EC_{50} <0.4 ppm) were detected after treatment with Myresa. Treatments with Imtrex and Revystar XE also resulted in the removal of bixafen sensitive isolates (isolates with EC_{50} < 0.3 ppm).

Figure 2. Fungicide sensitivity profiles of untreated and fungicide treated *Z. tritici* populations sampled at Perth (Dundee). Sampling was carried out 3-4 weeks after the T2 spray. Red vertical lines indicate sensitivity levels for mefentrifluconazole (0.05 and 0.4 ppm), prothioconazole-desthio (0.4 ppm), fentin chloride (0.25 ppm) and bixafen (0.3 ppm).



Unfortunately, only a few isolates were isolated from the trial in Telford. The results for the populations sampled at Cardigan (T1.5 spray) were similar to the Sutton Scotney populations, while the results from the populations sampled at Perth (T1 spray) resembled that from Caulshiel (data not shown).

Characterisation of fungicide insensitive *Z. tritici* strains isolated in 2021

All highly SDHI insensitive isolates (bixafen EC_{50} ≥ 5.0 ppm) from 2021 that were sequenced so far carried the succinate hydrogenase subunit C (SdhC) alteration H152R. No new Sdh variants were detected in 2021. Isolates with bixafen EC_{50} values between 1.0 and 5.0 ppm often carried Sdh mutations C-T79N, C-N86S or C-R151S and were also often less sensitive to fentin chloride, which is indicative of an enhanced efflux pump activity. An enhanced efflux pump activity can also further contribute to a decreased azole sensitivity as shown in Figure 3, where a selection of highly mefentrifluconazole insensitive isolates (EC_{50} ≥ 1.0 ppm) and/or prothioconazole-desthio insensitive isolates (EC_{50} ≥ 2.0 ppm) with information about their fentin chloride sensitivity and CYP51 sequences are shown.

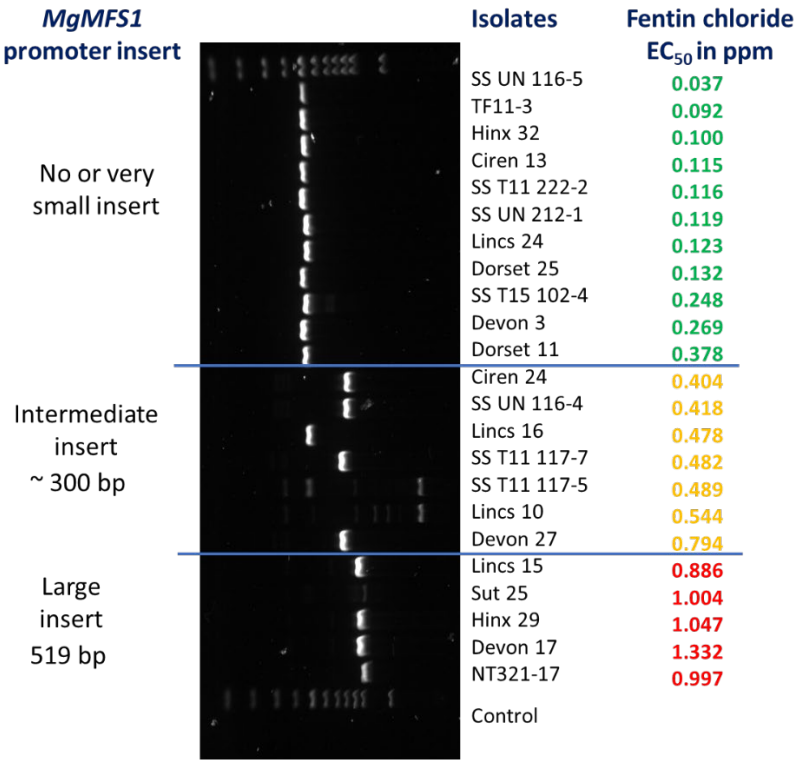
Figure 3. Characterisation of highly mefentrifluconazole and/or prothioconazole-desthio insensitive isolates. Isolates were ranked according their mefentrifluconazole EC_{50} values (low to high). High EC_{50} values for mefentrifluconazole (≥1.0 ppm), prothioconazole-desthio (≥2.0 ppm) and fentin chloride (≥0.4 ppm) are marked in yellow. Arrow indicates over-expression of CYP51.

Isolates	Mefentrifluconazole	Prothio-desthio	Fentin chloride	CYP51 variant
Zyatt 6	0.120	4.592	0.197	[L50S, V136A, S188N, A379G, I381V, Δ, N513K, S524T]
Dorset25	0.127	2.842	0.132	[L50S, D134G, V136A, A379G, I381V, Δ, S524T]
Devon 3	0.282	2.585	0.269	[L50S, D134G, V136A, S188N, A379G, I381V, Δ, S524T]
SS T11 117-7	0.484	2.692	0.482	[L50S, V136A, I381V, Y461H, S524T]
Lincs 16	0.491	2.000	0.478	[L50S, D134G, V136A, A379G, I381V, Δ, N513K, S524T]
CSH T15 11-6	0.552	2.002	0.180	[L50S, D134G, V136A, A379G, I381V, Δ, N513K, S524T]
CAR UNT 139-9	0.641	4.684	0.293	[L50S, V136A, S188N, A379G, I381V, Y461S, S524T]
SS T15 321-3	0.973	0.190	0.175	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]
Ciren 24	0.997	2.638	0.404	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]
Devon 27	1.212	0.311	0.794	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]
CSH T15 11-5	1.272	0.204	0.375	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]
Lincs 15	1.515	0.457	0.886	[L50S, V136C, S188N, I381V, Y461H, S524T]
TF T11-3	1.699	0.117	0.092	[L50S, S188N, A379G, I381V, A410T, Δ, N513K]
CSH T35 25-8	1.868	0.307	0.395	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]
CSH T35 25-3	1.967	0.343	0.390	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]
SS UN 116-4	2.032	0.634	0.418	[L50S, V136C, S188N, I381V, Δ, S524T]
Hinx 29	2.384	0.276	1.047	[L50S, S188N, I381V, Δ, N513K]↑
Devon 17	2.565	0.586	1.332	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]
Sut 25	6.263	0.678	1.004	[L50S, V136C, S188N, A379G, I381V, Δ, S524T]

The majority of the highly mefentrifluconazole insensitive isolates carried CYP51 variant [L50S, V136C, S188N, A379G, I381V, Δ, S524T]. V136C was present in all CYP51 variants, with exception of strains TF 11-3 and Hinx 29 carrying [L50S, S188N, A379G, I381V, A410T, Δ, N513K] and the CYP51 over-expressing [L50S, S188N, I381V, Δ, N513K], respectively. The highest levels of mefentrifluconazole insensitivity were measured for three isolates with the highest fentin chloride insensitivity (EC_{50} >1.0 ppm), which also includes strain TF 11-3. Although increased efflux pump activity can contribute to a reduction of azole sensitivity during *in vitro* growth, further *in planta* and/or field studies are needed to assess its impact in disease control. Because strain Hinx 29 is sensitive to fentin chloride, A410T, a rare CYP51 alteration but reported before, might contribute to a loss of mefentrifluconazole binding, but contributions of other resistance resistance mechanisms can not be ruled out. High levels of prothioconazole-desthio insensitivity were linked with the presence of V136A in complex CYP51 variants that are also harbouring I381V and S524T among other alterations.

MgMFS1 is one of the efflux pumps that have been reported to impact on fungicide sensitivity in lab derived mutants of *Z. tritici* when its expression is altered. Constitutive overexpression of this transporter belonging to the Major Facilitator Superfamily (MFS) due to a 519 bp promoter insert has been reported to affect fungicide sensitivity to DMI, QoI and SDHI fungicides in field isolates. More recently, various other *MgMFS1* promoter inserts of 150, 267, 338, 369 and 377 bp have also been reported with weaker effects on increased efflux activity and fungicide sensitivity. PCR was used to check if there is a correlation between the presence of differently sized *MgMFS1* promoter inserts and fentin chloride sensitivity. Figure 4 shows there is a good correlation between *MgMFS1* promoter inserts and fentin chloride sensitivity. A large insert of 519 bp is present in isolates with the highest fentin chloride EC_{50} values >0.8 ppm, which includes isolates Devon 17, Hinx 29, Sut 25, Lincs 15 and the reference isolate NT321.17. Smaller insert of approximately 300 bp are present in most moderate insensitive isolates showing EC_{50} values between 0.4 and 0.8 ppm and no or very small inserts are present in sensitive isolates showing EC_{50} values between 0.04 and 0.4 ppm. PCR amplicons will be sequenced to establish the exact size of the *MgMFS1* promoter inserts in a selection of isolates. Isolates showing EC_{50} values ≥0.25 ppm for fentin chloride are regarded as insensitive and in the absence of promoter inserts other alterations associated MgMFS1 expression or substrate binding as well as other efflux pumps might be involved.

Figure 4. Correlation between fentin chloride sensitivity and presence of *MgMFS1* promoter inserts in *Z. tritici* field isolates using PCR analysis. Isolate NT321.17 contains 519 bp *MgMFS1* promoter insert and was included as control. 100 bp ladder was used as marker in gel electrophoresis to esimate PCR amplicon size.



Azole sensitivity profiling of early season *Z. tritici* field populations in 2022

Figure 5A shows the prothioconazole-desthio sensitivity profiles of untreated early season *Z. tritici* populations at Rothamsted (Harpenden, Hertfordshire) during 2003-2022. The prothioconazole-desthio sensitivity profile of 2022 (n=37) was similar than that of the population sampled in 2021 (n=54), although the frequency of insensitive sensitive strains (EC_{50} ≥ 0.4 ppm) was higher in 2022 than 2021, with 56 and 35 %, respectively. Among the five different populations tested (Figure 5B), the population sampled in Leicestershire was most sensitive, while the population from Kent was least sensitive.

Figure 5. Prothioconazole-desthio sensitivity profiles of untreated early season *Z. tritici* populations sampled at Rothamsted (Hertfordshire) over time (A) and different locations (B) in Cambridgeshire, Devon, Kent and Leicestershire during February-March in 2022. Red vertical line indicates prothioconazole-desthio sensitivity level of 0.4 ppm.

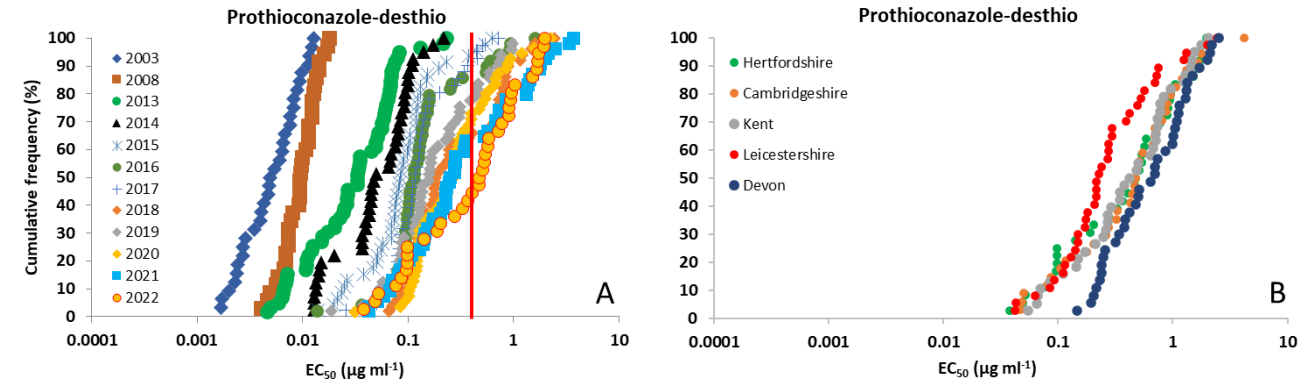
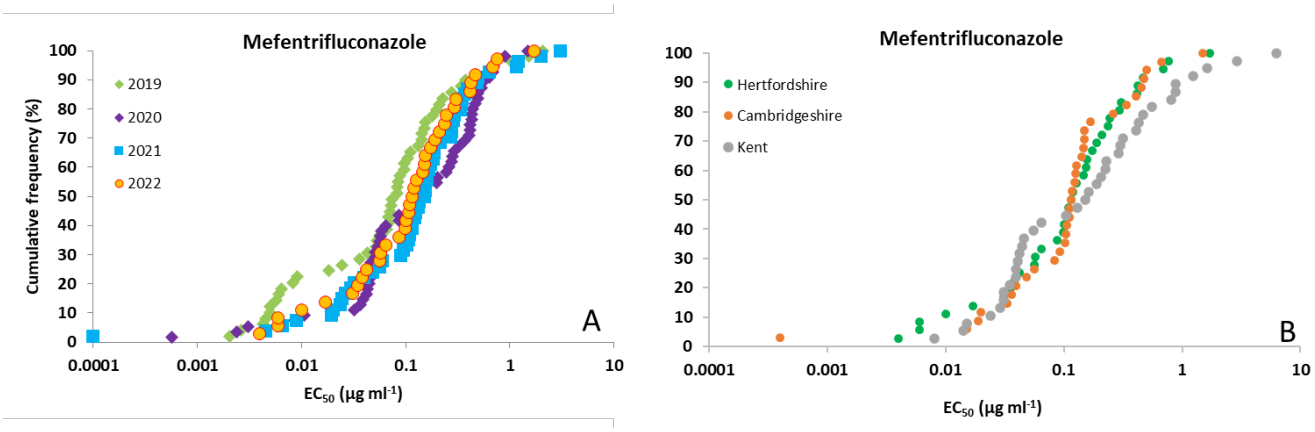


Figure 6A shows that the mefentrifluconazole sensitivity of the 2022 Rothamsted population (Hertfordshire) was almost identical to the population sampled in 2021. All three different populations tested (Cambridgeshire, Hertfordshire and Kent) showed a similar sensitivity profile, with the majority of isolates showing EC_{50} values between 0.01 and 1.0 ppm (Figure 6B).

Figure 6. Mefentrifluconazole sensitivity profiles of untreated early season *Z. tritici* populations sampled at Rothamsted (Hertfordshire) during 2019-2022 (A) and at different locations (B) in Cambridgeshire, Hertfordshire and Kent during February-March in 2022.

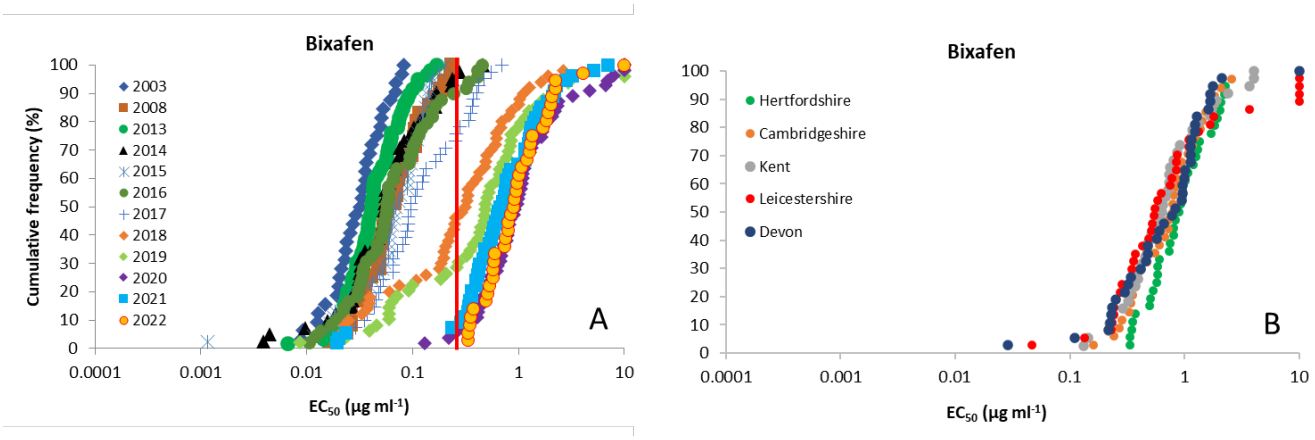


Annual Project Report 2022

SDHI sensitivity profiling of early season *Z. tritici* field populations in 2022

Regarding bixafen, which shows high levels of cross-resistance to other SDHIs, including fluxapyroxad, penthiopyrad, isopyrazam, and benzovindiflupyr, the sensitivity profile of the Rothamsted 2022 population was similar to the 2021 population. Sensitive isolates (EC_{50} < 0.3 ppm) were absent in 2022, while only one strain showing EC_{50} values ≥10 ppm (Figure 7A).

Figure 7. Bixafen sensitivity profiles of untreated early season *Z. tritici* populations sampled at Rothamsted (Hertfordshire) over time during 2003-2022 (A) and at different locations (B) in Cambridgeshire, Devon, Hertfordshire, Kent and Leicestershire during February-March in 2022.

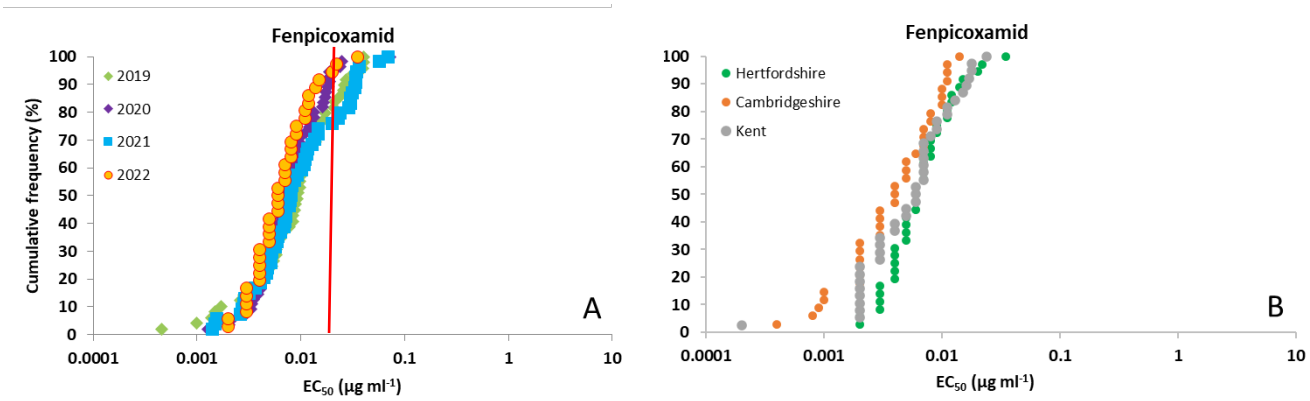


Similar bixafen sensitivity profiles were recorded for all five populations sampled at different locations (Figure 7B), with only a few isolates sensitive to bixafen (EC_{50} < 0.3 ppm). Most highly insensitive isolates for bixafen (EC_{50} values ≥10 ppm) were detected in Leicestershire with 5 out of 37 isolates (14 %), while no highly insensitive isolates were found in the Kent population (n=38).

Fenpicoxamid sensitivity profiling of early season *Z. tritici* field populations in 2022

Sensitivity profiles were also generated for fenpicoxamid, a new QiI fungicide recently introduced on to the UK cereal market for Septoria leaf blotch control. The *Z. tritici* populations sampled at Rothamsted over time during 2019-2022 were extremely sensitive to fenpicoxamid (Inatreq) with all isolates showing EC_{50} values <0.1 ppm (Figure 8A). The 2021 population was least sensitive with approximately 20 % of the population showing EC_{50} values between 0.02 and 0.1 ppm. Similar fenpixoxamid sensitivity profiles were recorded for the three different populations tested that were sampled in Cambridgeshire, Hertfordshire and Kent (Figure 8B).

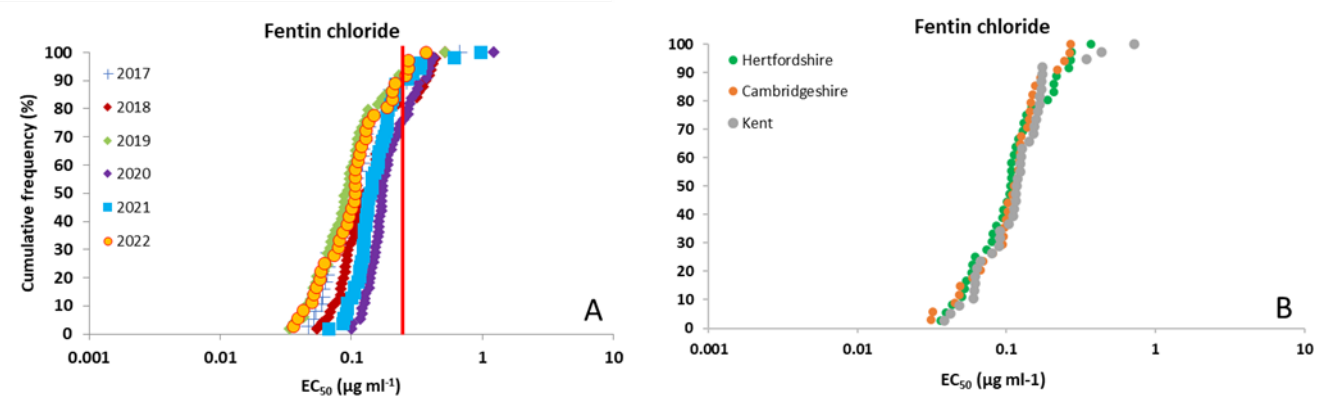
Figure 8. Fenpicoxamid sensitivity profiles of untreated early season *Z. tritici* populations sampled at Rothamsted (Hertfordshire) over time during 2019-2022 (A) and at different locations in Cambridgeshire, Hertfordshire and Kent during February-March in 2022 (B). Red vertical line indicates fenpicoxamid sensitivity level of 0.02 ppm.



Fentin chloride sensitivity profiling of early season *Z. tritici* field populations in 2022

Figure 9 shows the results of sensitivity testing using the organotin fungicide fentin (triphenyl tin) chloride, an ATP synthase inhibitor, which can also act as a substrate for different fungal efflux pumps.

Figure 9. Fentin chloride sensitivity profiles of untreated early season *Z. tritici* populations sampled at Rothamsted (Hertfordshire) over time during 2017-2022 (A) and at different locations in Cambridgeshire, Hertfordshire and Kent during 2022 (B). Red vertical line indicates fentin chloride sensitivity level of 0.25 ppm.



Annual Project Report 2022

The fentin chloride sensitivity profile at Rothamsted has not changed much during 2017-2022, a higher proportion of insensitive isolates (EC_{50} values ≥0.25 ppm) were recorded in 2018 and 2020, but the frequency of highly insensitive isolates (EC_{50} values ≥1.0 ppm) due to the 519 bp *MgMFS1* promoter insert remains low (<2 %) (Figure 9A). Similar fentin chloride sensitivity profiles were recorded for the three different populations sampled in Cambridgeshire, Hertfordshire and Kent during early season in 2022 (Figure 9B), with no highly insensitive isolates detected.

Key issues to be addressed in the next year

- Isolation of strains from late season samples of the fungicide performance trials from untreated plots and plots treated with Myresa, Revystar XE and Univoq
- Fungicide sensitivity testing of isolates using mefentrifluconazole, prothioconazole-desthio, bixafen, pydiflumetofen, fenpicoxamid and fentin chloride
- Additional compounds will be screened against the Rothamsted population for baseline studies/cross-resistance studies, these include the SDHIs fluopyram, isoflucypram and fluindapyr, and the new QoI metyltetraprole
- Characterisation of fungicide insensitive strains (target & non-target fungicide resistance mechanisms)
- Development of next generation PCR amplicon sequencing platforms to detect SdhC & CYP51 mutations

Lead partner	NIAB
Scientific partners	ADAS, SRUC and Teagasc
Industry partners	BASF, Bayer CropScience, Corteva and Syngenta
Government sponsor	

Has your project featured in any of the following in the last year?	
Events	***Press articles***
	'Tracking fungicide resistance' by Arable Farming Magazine April 2022 issue
Conference presentations, papers or posters	***Scientific papers***

Annual Project Report 2022

Results of the project were used in the presentation of the fungicide performance trials at the AHDB Agronomists' Conference in 2021	Hellin P, Duvivier M, Heick TM, Fraaije BA, Bataille C, Clinckemaillie A, Legrève A, Jørgensen LN, Anderson B, Samils B, Rodemann B, Berg G, Hutton F, Garnault M, Jarroudi ME, Couleaud G & Kildae S Hellin (2021) 'Spatio-temporal distribution of DMI and SDHI fungicide resistance of *Z. tritici* throughout Europe based on frequencies of key target-site alterations. *Pest Management Science* 12, 5576-5588
Other	
Presentations • 'SDHI and DMI fungicide sensitivity testing of UK *Zymoseptoria tritici* field populations' at on-line AHDB fungicide performance trials project meeting (28 January 2022) • 'Update on azole and SDHI resistance in UK populations of *Zymoseptoria tritici'* at on-line FRAG-UK meeting (9 March 2022) • 'SDHI and DMI fungicide sensitivity testing of UK *Zymoseptoria tritici* field populations' EU Agronomist extension meeting (10 March 2022) • Presentations at on-line meetings with AgroChem industry partners (ADAMA, BASF, Corteva and UPL)	