Annual Project Report 2021

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

This project, which is linked to the AHDB winter wheat fungicide performance trials, 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 targeting 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 objectives for this project 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 base-line 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 and 5 Knowledge transfer of the fungicide sensitivity status of septoria and other key cereal pathogens to assist resistance management.

Key messages emerging from the project

- No significant further shifts in azole insensitivity 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 and further monitoring is required to understand further adaption.
- Further shifts in SDHI insensitivity (removal of sensitive strains) and slow accumulation of C-H152R strains in populations is ongoing. Other highly resistant Sdh variants (B-H267L, C-N86K and B-N225T+C-N86S) are rarely detected, indicating a fitness cost is also likely associated with these variants.

- Monitoring of baseline sensitivities to new actives for septoria leaf blotch control likely to enter the market, including fenpicoxamid (QiI), and the SDHIs pydiflumetofen and isoflucypram, is ongoing and have been carried out for all populations sampled in 2020.

Summary of results from the reporting year

Azole sensitivity shifts in field populations over time. Figure 1 shows the prothioconazole-desthio sensitivity profiles of untreated early season *Z. tritici* populations at Rothamsted (Harpenden, Hertfordshire). The prothioconazole-desthio sensitivity profile of 2021 was similar than that of populations sampled after 2017, although a higher frequency (20 %) of highly insensitive sensitive strains (EC_{50} >1 ppm) was measured in 2021 (n=54).

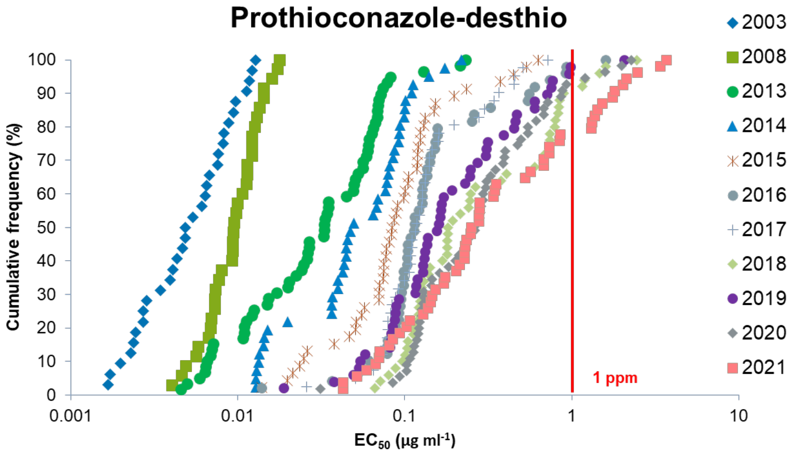


Figure 1. Prothioconazole-desthio sensitivity profiles of *Z. tritici* populations sampled at Rothamsted over time.

Fourteen different CYP51 variants were detected in 46 strains of the 2021 population, with 11 out of 14 variants carrying S524T (83 %). Frequency of V136C is also increasing, with 46 % of strains testing positive. Figure 2 shows the frequencies of the most common CYP51 variants (detection levels ≥ 5%) in *Z. tritici* field populations at Rothamsted sampled over time. The most common variant was [L50S, V136C, S188N, A379G, I381V, DEL, S524T] (33 %) followed by the over-expressing variant [L50S, I381V, S188N, DEL, N513K] (13 %), [L50S, V136C, S188N, I381V, Y461H, S524T] (11 %) and [L50S, D134G, V136A, A379G, I381V, DEL, N513K, S524T] (11 %).

Annual Project Report
2021

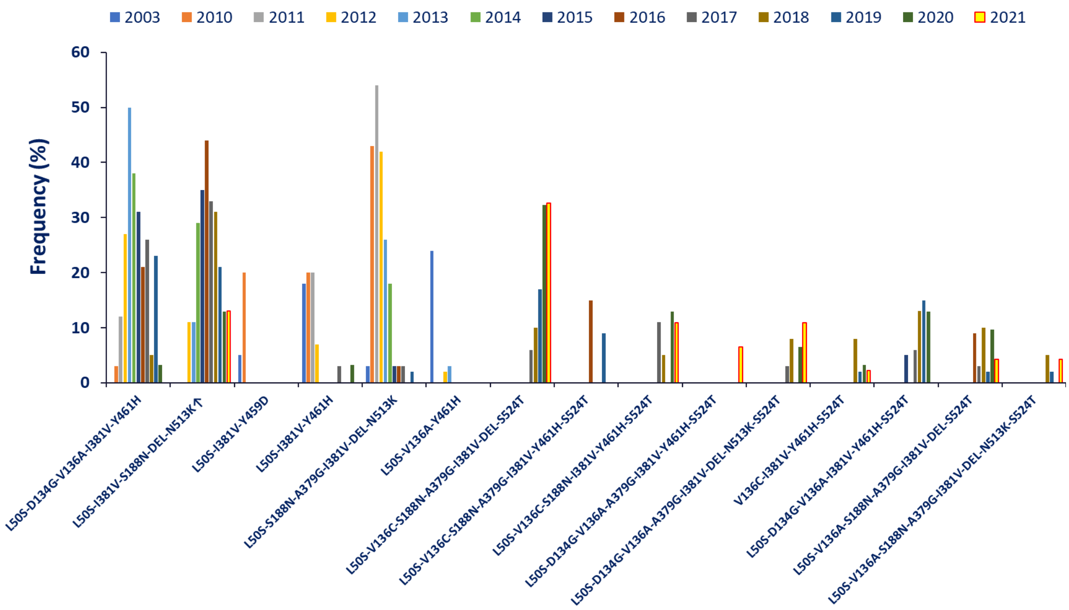


Figure 2. Dynamics of CYP51 variants in Z. *tritici* populations sampled at Rothamsted. DEL: deletion of 6 bp resulting in the loss of two amino acids (Y459 and G460); ↑ indicates *CYP51* overexpression.

The baseline sensitivity for metrifluconazole was also established for all archived Rothamsted populations sampled in 2019-2021 after the market introduction of Revysol in spring 2020 (Figure 3).

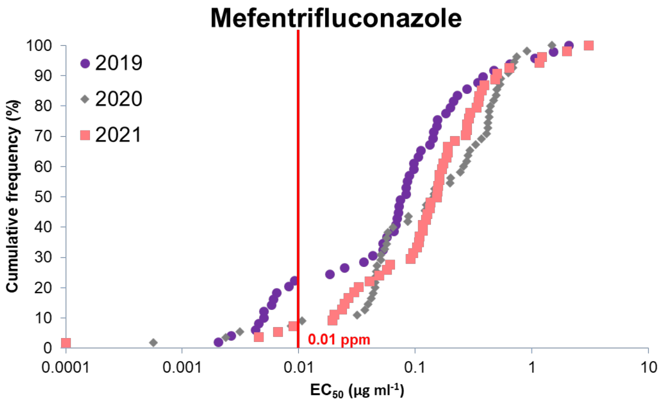


Figure 3. Mefentrifluconazole sensitivity profiles of Z. *tritici* populations sampled at Rothamsted.

In comparison with 2019, less extreme mefentrifluconazole sensitive strains (EC_{50} <0.01 ppm) were detected in 2020 and 2021. All extreme mefentrifluconazole sensitive isolates from 2019 carried CYP51 variant [L50S, D134G, V136A, I381V, Y461H]. The most insensitive variant in 2019 was [L50S, V136C, S188N, A379G, I381V, DEL, S524T], followed by over-expressing [L50S, I381V, S188N, DEL, N513K] and [L50S, D134G, V136A, I381V, Y461H, S524T], respectively. The selection is different for prothioconazole-desthio with the over-expressing [L50S, I381V, S188N, DEL, N513K] as the most sensitive and [L50S, D134G, V136A, I381V, Y461H, S524T] as the least sensitive CYP51 variant, followed by [L50S, V136C, S188N, A379G, I381V, DEL, S524T] and [L50S, D134G, V136A, I381V, Y461H], respectively. The ranking of CYP51 variants according to sensitivity to different azoles was more complex in 2020 and 2021 due to presence of more variants at low frequencies and higher frequencies of fentin chloride insensitive strains (EC_{50} ≥0.15 ppm) indicating increased levels of efflux pump activity that in combination with CYP51 alterations reduces the azole sensitivity further.

SDHI sensitivity shifts in field populations over time. Regarding bixafen, which shows high levels of cross-resistance to fluxapyroxad, penthiopyrad, isopyrazam, and benzovindiflupyr, the sensitivity profile of the 2021 population was similar to the 2020 population, with low frequencies recorded for both sensitive (EC_{50} >0.3 ppm) and highly resistant strains (EC_{50} >10 ppm).

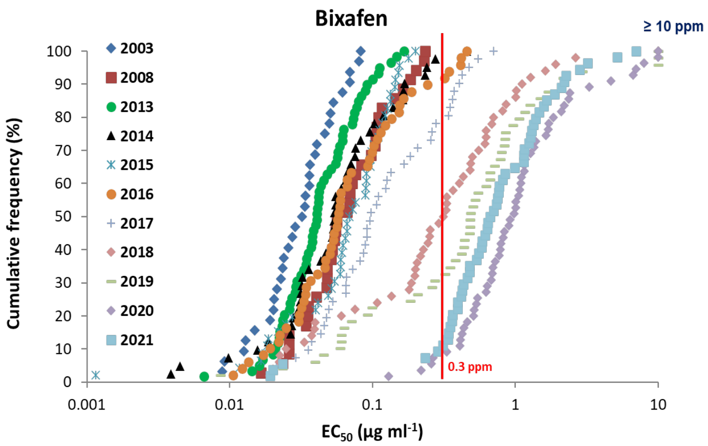
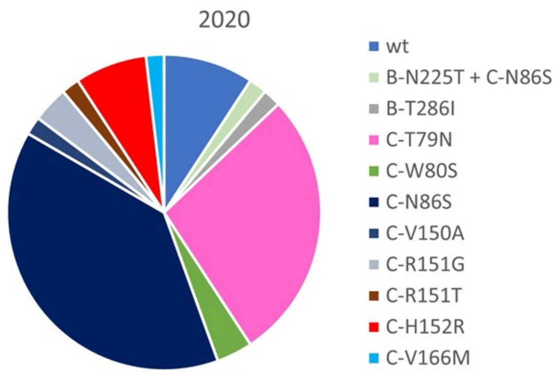


Figure 4. Bixafen sensitivity profiles of untreated early season *Z. tritici* populations sampled at Rothamsted over time.

Sdh genotyping of Rothamsted field isolates confirmed the phenotyping results showing that approximately 10 % of *Z. tritici* strains within the field population carry no key Sdh alterations known to affect the binding of SDHI fungicides in both 2020 and 2021.

Annual Project Report
2021

Half of the 2021 population consisted of strains carrying SdhC N86S, followed by C-T79N (18 %), C-R151S (8 %) and both C-V166M and C-H152R (5 %). Of these SdhC variants, only C-H152R confers high levels of insensitivity to SDHI fungicides (EC_{50} ≥5.0 ppm) but this variant is only slowly accumulating in field populations due to a previously reported fitness cost associated with this alteration.



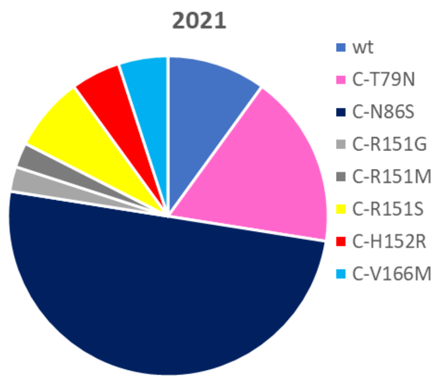


Figure 5. Distribution of Sdh variants within *Z. tritici* populations sampled at Rothamsted in 2020 (n=54) and 2021 (n=40)

Fungicide sensitivity shifts in the wheat fungicide performance trials. Trials sampled at Sutton Scotney (Hampshire), Cardigan (Wales) and Cauldshiel (East Lothian) yielded enough strains to study the effects of a selection fungicide treatments based on solo products (Proline 275 (a.i. prothioconazole)) and Imtrex (a.i. fluxapyroxad)) or mixtures (Revystar XE (fluxapyroxad + mefentrifluconazole) and Ascra Xpro (bixafen + fluopyram + prothioconazole)). There were no significant differences in the fungicide sensitivity profiles of the populations sampled at the different locations at the start of the season. No shift in prothioconazole-desthio sensitivity was measured in field populations sampled three weeks after treatments at all three locations. A significant effect on bixafen sensitivity, selection for highly resistant strains, was only measured at Cardigan, while at this location and to a lesser extent at Sutton Scotney only small shift in mefentrifluconazole sensitivity was observed (Figure 6). The lack of selection can be explained by an already fungicide-adapted field population and/or low disease pressure at most locations, caused by significant below average rainfall during March-May in 2020 in both England and Scotland. March-May is the key period for asexual spore production and splash dispersal by rain and fungicide are applied in this period to control Septoria at T1 (leaf 3 fully emerged) and T2 (flag leaf fully emerged).

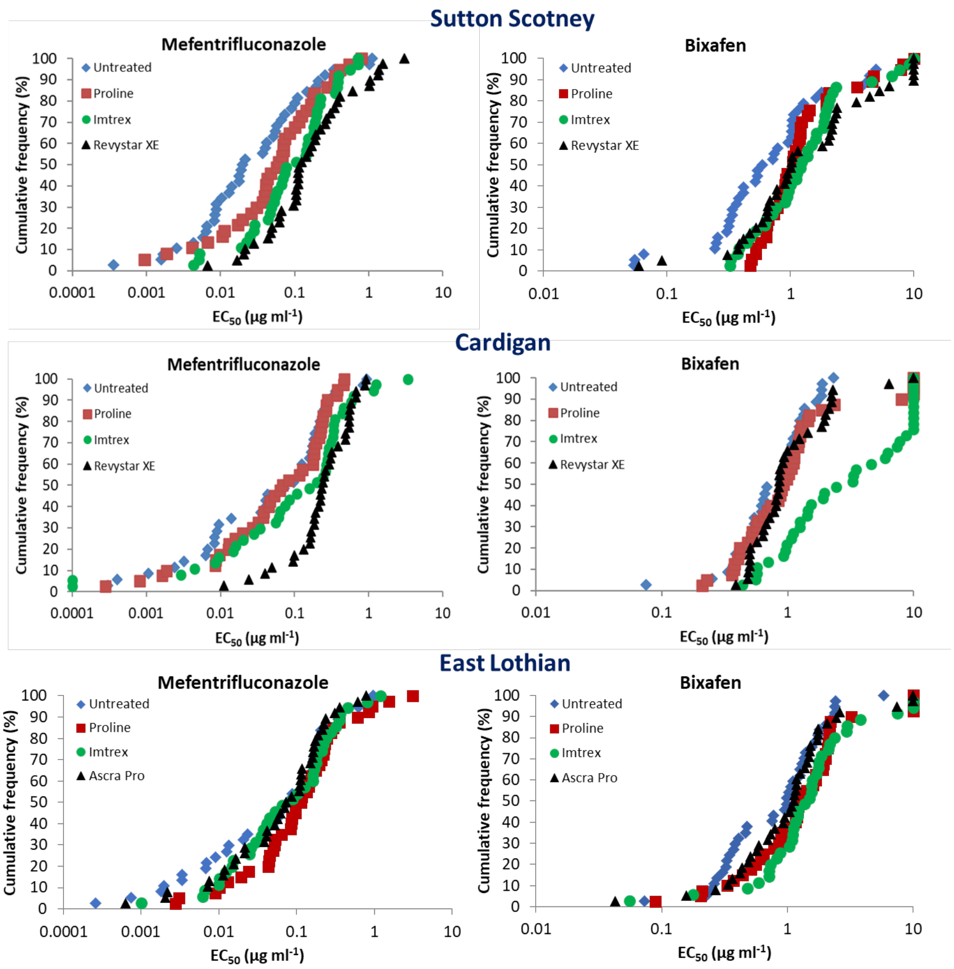


Figure 6. Mefentrifluconazole (left) and bixafen (right) sensitivity profiles of *Z. tritici* populations sampled from untreated and treated plots at Sutton Scotney (top), Cardigan (middle) and East Lothian (bottom) in 2020. Crops at Sutton Scotney (T1 with cv. Gravity) and Cardigan (T2 with cv. Elation) received a protectant fungicide application, while at East Lothian (T2 with cv. Viscount) fungicide was applied to provide both protectant and eradicant disease control. Treatments were Proline 275 (0.72 L/ha) and Imtrex (2.0 L/ha), while Revystar XE or Ascra Xpro were both applied at 1.5 L/ha. Sampling of infected leaves for strain isolation was carried out approximately three weeks after treatment.

As expected, treatment with Imtrex (solo SDHI) in comparison with the other treatments resulted in selection for highly bixafen-insensitive strains (40 % of strains showing EC_{50} >5.0 ppm) at Cardigan. At

the other locations, highly bixafen-insensitive strains carrying C-H152R were only detected at low levels (<10 %). The application of Revystar XE didn't result in selection of highly bixafen insensitive strains at Cardigan because mefentrifluconazole is an effective SDHI mixing partner. Although Revystar XE didn't select for highly mefentrifluconazole-insensitive strains (EC_{50} values >1.0 ppm) in comparison with other treatments (untreated included) at Cardigan, mefentrifluconazole-sensitive strains (EC_{50} values <0.01 ppm) were effectively removed from the population after treatment. A similar trend, albeit less pronounced, was also observed for populations sampled at Sutton Scotney. **Fungicide base-line sensitivity testing.** Monitoring of base-line sensitivities to fenpicoxamid (QiI), and the SDHIs pydiflumetofen and isoflucypram, all new actives for Septoria leaf blotch control likely to enter the market soon, was carried out for all field populations sampled in 2020 and will continue in 2021.

Key issues to be addressed in the next year
Choice of fungicides to be included in sensitivity testing and decision making on which treatments to sample from the wheat fungicide performance trials in consultation with the Fungicide Working Group and scientific partners.

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***
Conference presentations, papers or posters	***Scientific papers***
Results of the project were used in the presentation of the Fungicide Performance trials at the AHDB Agronomists' Conference in 2020	
Other	
Presentations at Fungicide Working Group (14/10/20 and 29/01/21) and FRAG-UK meeting (18/11/20) and seminar at NIAB 'Chemical control of Septoria leaf blotch: to predict the future look to the past' (03/06/20). Additional meetings with several Industry partners.	