Annual Project Report
April 2023 to March 2024

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 2026

Project aim and objectives

Linked to the AHDB winter wheat fungicide performance trials, this project establishes baseline sensitivities for new actives entering the market and monitors shifts in sensitivity (phenotype-to genotype relationships) in UK *Zymoseptoria tritici* (Zt) (septoria tritici) populations to all key fungicides belonging to different mode of actions (MOAs). In addition, DNA-based diagnostic assays that target new genotypes are 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 four objectives are:

1. Measure in vitro sensitivity of untreated, early-season septoria field populations sampled at different locations in the UK and Ireland to the key fungicide classes: azoles, SDHIs and QiIs. Compare these with available baseline sensitivities of populations sampled in previous seasons.
2. Measure the effect of different spray programmes on fungicide sensitivity shifts in septoria populations sampled in the AHDB wheat 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 and characterise cross-resistance profiles associated with the key resistant genotypes.
4. Transfer knowledge of the fungicide sensitivity status of septoria (and other key cereal pathogens) in order to devise and disseminate strategies based on appropriate fungicide inputs and a more sustainable disease management by minimising fungicide resistance development.

Annual Project Report
April 2023 to March 2024

Key messages emerging from the project

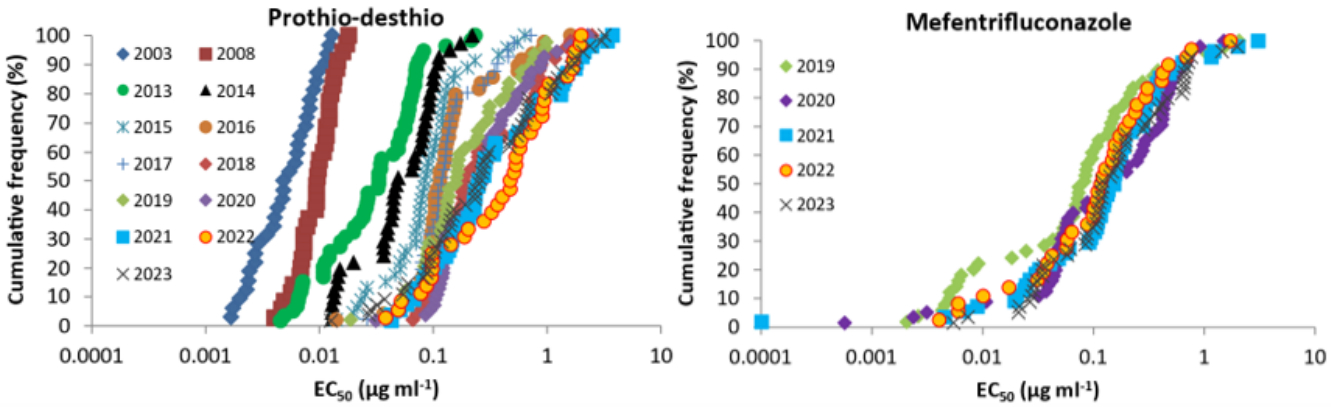
- Continued slide in azole sensitivity (prothioconazole-desthio and mefentrifluconazole) in septoria due to shifts in frequency within the known range, but no isolates beyond the previous upper limit. CYP51 genotyping of less-sensitive isolates is currently underway.
- Mefentrifluconazole cross-resistance with prothioconazole-desthio is incomplete, and the relative impacts of prothioconazole or mefentrifluconazole treatment on the sensitivity of late-season isolates differs between trial sites. Further genetic studies are needed to determine which CYP51 mutations are selected by which compound.
- Further shifts in SDHI insensitivity have been observed, with increasing frequencies of isolates at the lowest measured sensitivity to bixafen from multiple locations, and partial cross-resistance between pyrazole-4-carboxamides (bixafen) and a new stretched heterocycle amide (SHA)-SDHI compound.
- The least sensitive isolates have sdhC-H152R, and an isolate has been found with sdhC-H152R in combination with sdhB-I269V (2022) and with sdbB-H267N (2023). Other mutations such as sdhC-T79N and sdhC-N86S continue to be found in intermediate-sensitivity isolates, or in combination with other mutations such as sdhB-I269V in less sensitive double mutants.
- Fenpicoxamid sensitivity range of 2023 isolates remained within the previously established baseline, with slightly (<5-fold) lower average EC_{50} in QiI treated samples from some sites.
- Monitoring of baseline sensitivities to new actives for septoria control likely to enter the market is ongoing (e.g. pydiflumetofen, fluindapyr, and metyltetrapole) and will be used to detect fungicide sensitivity shifts as soon as new products enter the market. Isoflucypram is now available for 2024 and isoflucypram-treated plots will be sampled in 2024.
- Enhanced efflux pump activity in some septoria isolates has reduced the sensitivity to azole, QoI, QiI and SDHI fungicides during *in vitro* growth. Further studies are needed to assess the impact during an *in planta* growth in the glasshouse and in field trials, as well as ongoing monitoring of the frequency in the *Z. tritici* population and in combination with different target-site alterations.

Summary of results from the reporting year

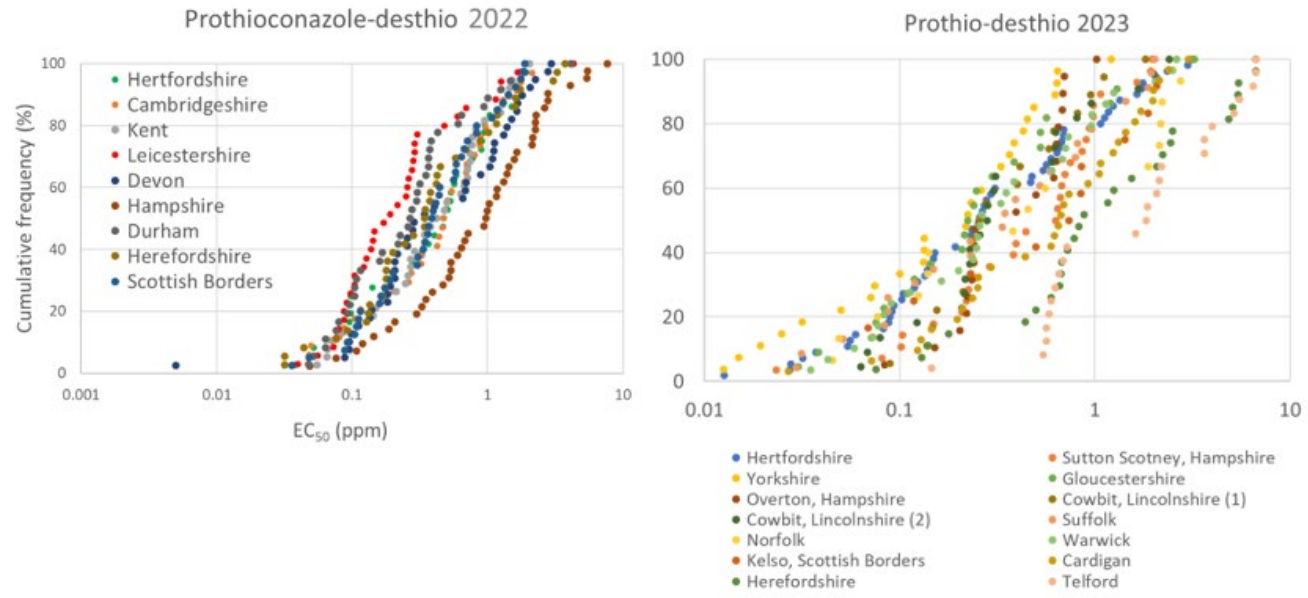
Azole sensitivity

Early-season 2023 isolates from Rothamsted, Hertfordshire had similar prothioconazole sensitivity (prothio-desthio metabolite used for in vitro testing) to 2021, and similar mefentrifluconazole sensitivity to 2020-2023.

Annual Project Report
April 2023 to March 2024

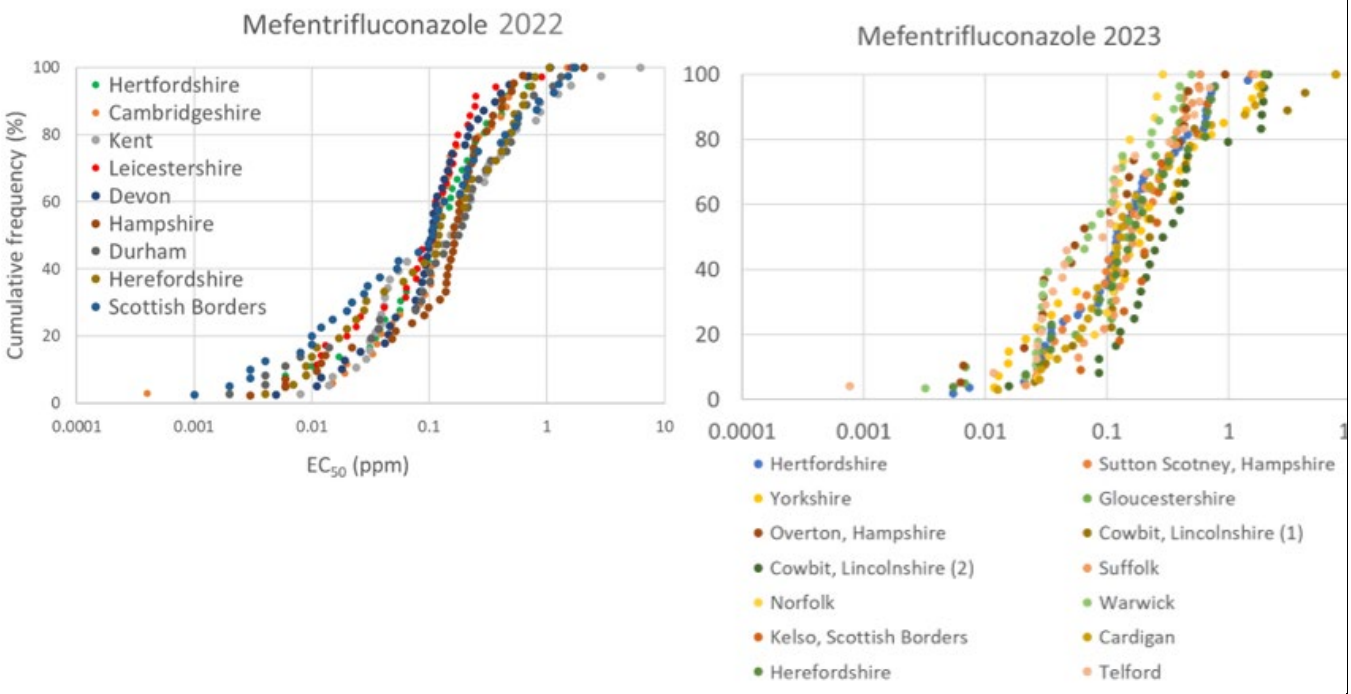


In 2022 early season samples, the lowest prothioconazole sensitivity was seen in isolates from Sutton Scotney, Hampshire. In 2023, the Sutton Scotney isolates had similar sensitivity to 2022, but lower sensitivity was seen in samples from Telford, Herefordshire and Norfolk. Other sites were within the range seen in 2022. Sequencing of the *CYP51* gene from selected less-sensitive isolates is currently in progress.



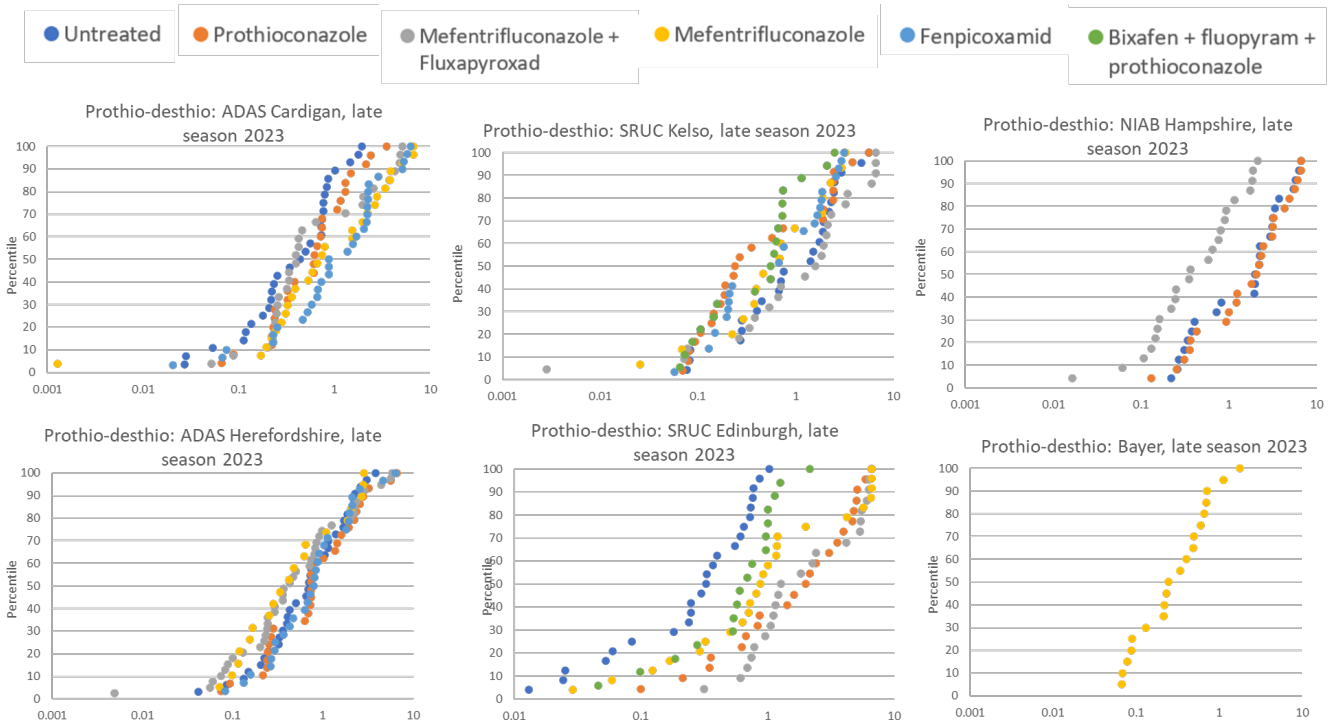
Annual Project Report
April 2023 to March 2024

Mefentrifluconazole sensitivity was broadly within the range seen in 2022, but with some less sensitive isolates found in Cardigan, Lincolnshire and Yorkshire. Sequencing of the *CYP51* gene from these isolates is currently in progress. Overall, there was a reduction in frequency of isolates with EC_{50} < 0.01 µg ml^{-1}, and an increase in frequency of isolates with EC_{50} 0.1-1 µg ml^{-1}.



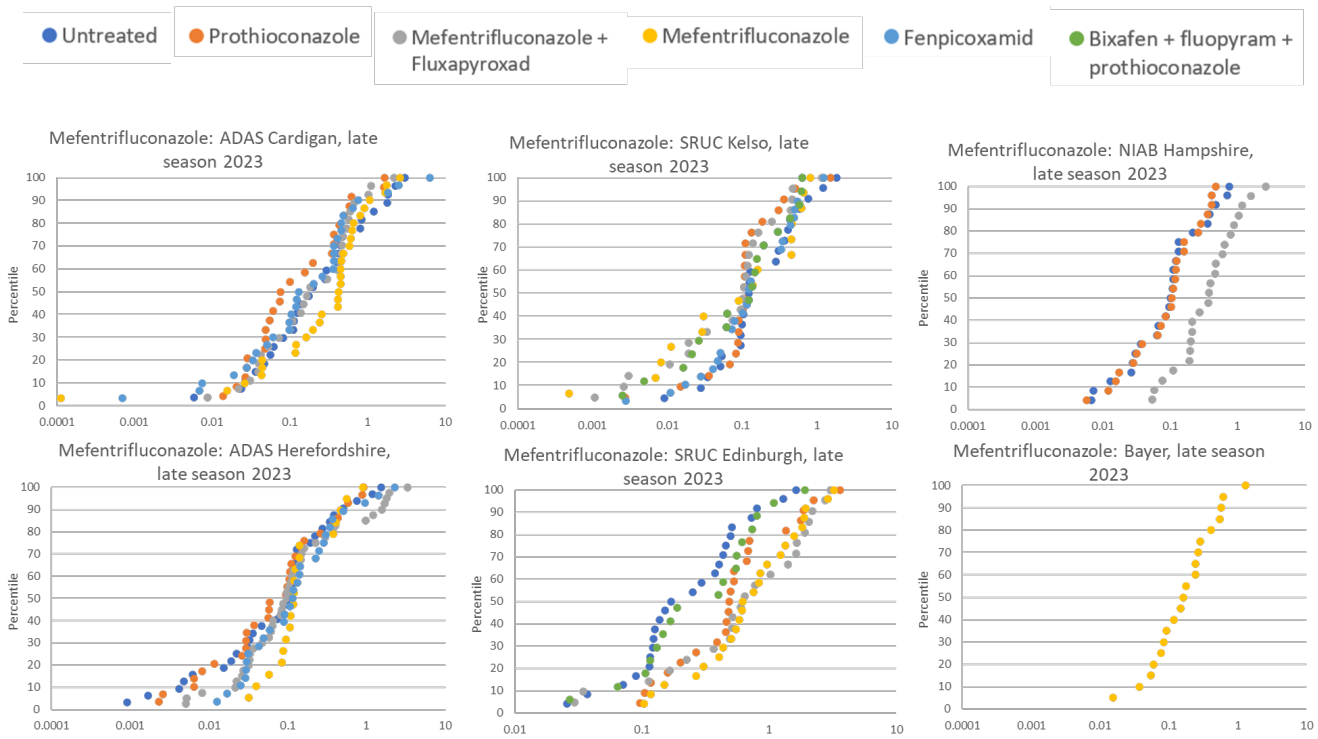
Late-season isolates showed varying patterns of azole selection across different trial sites. Prothioconazole sensitivity was lower in prothiooncazole- and mefentrifluconazole-treated plots from the SRUC Edinburgh site, but lower in prothioconazole-treated and untreated plots than in mefentrifluconazole+fluxapyroxad related plots from the NIAB Sutton Scotney site in Hampshire. For other sites, sensitivity was similar across the tested treatments.

Annual Project Report
April 2023 to March 2024



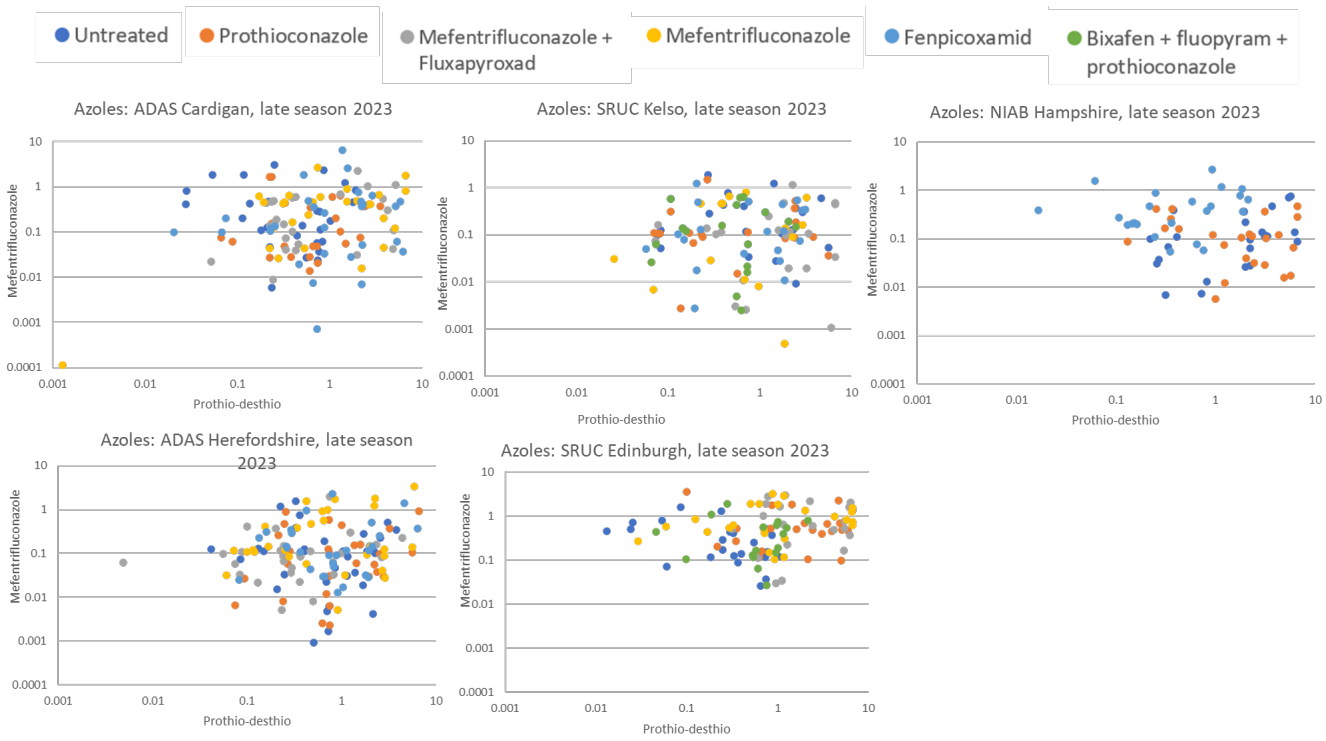
In the samples from ADAS Cardigan and ADAS Herefordshire sites, mefentrifluconazole treatment appeared to result in some selection again of the more sensitive isolates in the population, but this was not seen for the SRUC Kelso site. The solo mefentrifluconazole and mefentrifluconazole+fluxapyroxad treated samples from the SRUC Edinburgh site and the mefentrifluconazole+fluxapyroxad-treated sample from the NIAB Sutton Scotney, had reduced mefentrifluconazole sensitivity across the range. Prothioconazole-treated samples from SRUC Kelso and SRUC Edinburgh sites also had reduced frequencies of more mefentrifluconazole-sensitive isolates, but without an increase in the least-sensitive isolates.

Annual Project Report
April 2023 to March 2024



Comparing prothioconazole and mefentrifluconazole sensitivity indicated weak to no cross-resistance within the isolates present in 2023, although longer-term data shows some positive correlation. However, there are some isolates with the lowest sensitivity to both azoles and no clear evidence of negative cross-resistance. Therefore, there remains a risk of selecting isolates with reduced sensitivity to both azoles, and so they should continue to be used with non-azole mixing partners.

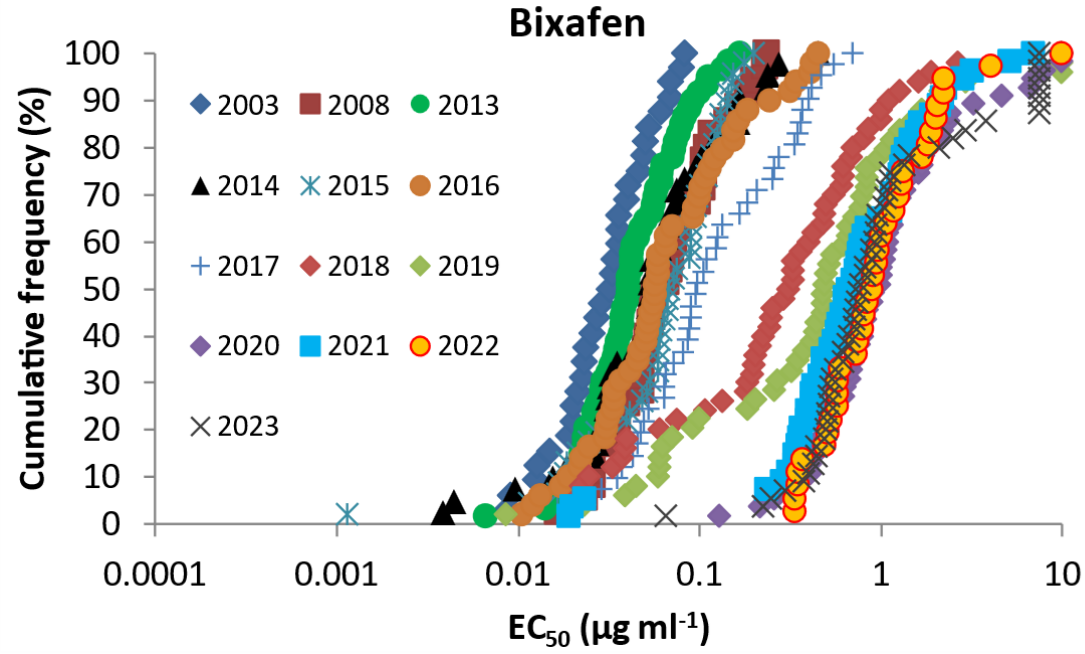
Annual Project Report
April 2023 to March 2024



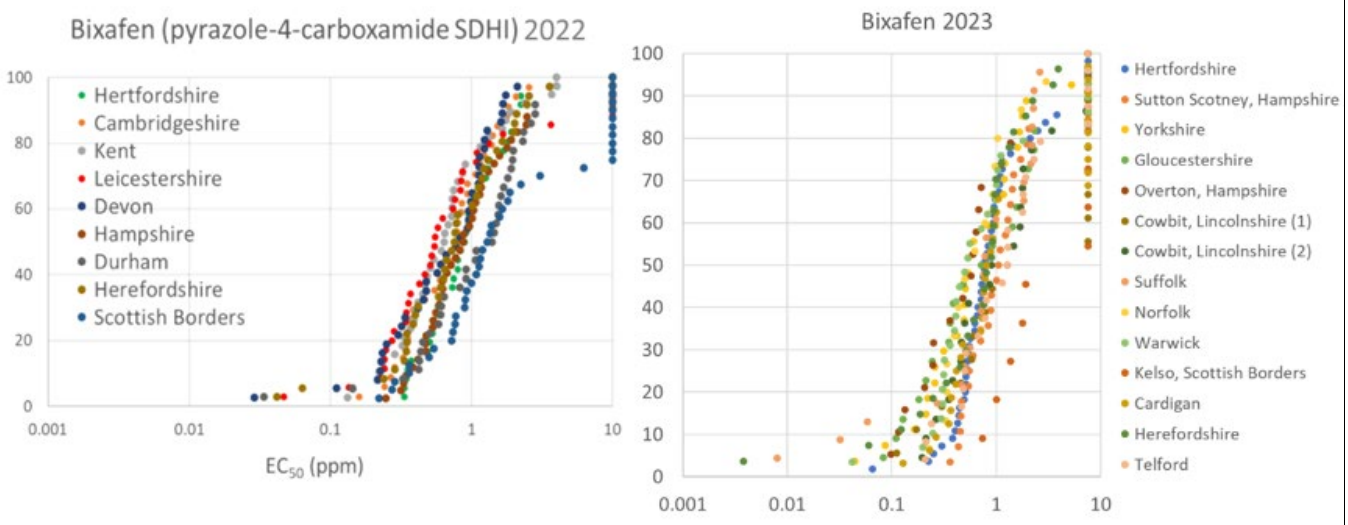
SDHI sensitivity

Early-season isolates from Rothamsted (Hertfordshire) showed an increase in frequency of the least bixafen-sensitive isolates compared to previous years, while the median remained similar to 2021-2023.

Annual Project Report
April 2023 to March 2024



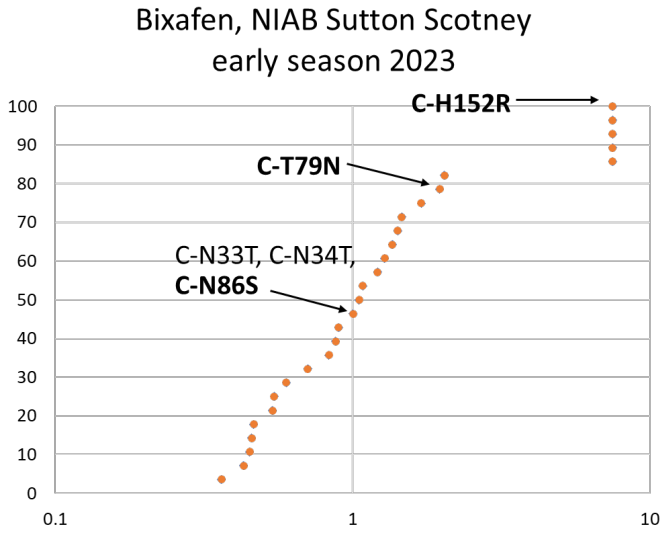
Early-season samples from different sites showed a similar increase in least sensitive isolates from multiple locations, whereas in 2022 this was seen only at the SRUC Scottish Borders site.

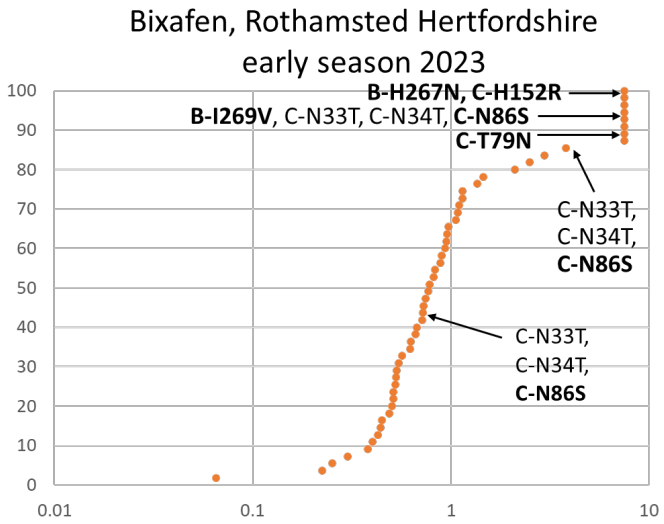


The results described in this summary report are interim and relate to one year. In all cases, the reports refer to projects that extend over a number of years.

Annual Project Report
April 2023 to March 2024

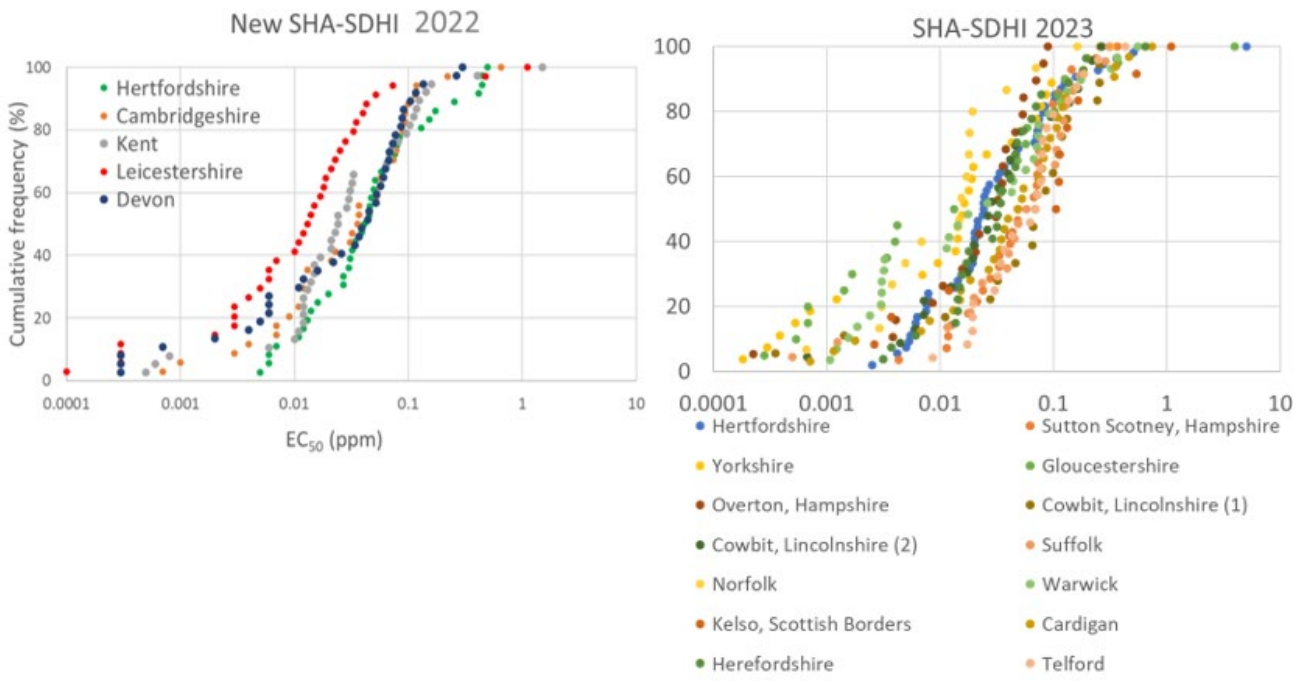
Sequencing of selected isolates showed that, as seen in previous years, sdhC-N86S or sdhC-T79N single mutants were moderately resistant, whereas double mutants or sdhC-H152R containing genotypes were highly resistant. One highly resistant isolate contained a single sdhC-T79N mutation, and further testing will ascertain whether non-target-site mechanisms such as efflux resulted in lower-than-expected sensitivity for this genotype. In 2022, one least sensitive isolate was an H152R double mutant, with sdhB-I269V + sdhC-H152R. In 2023 early season isolates, an additional H152R double mutant was found, with sdhB-H267N + sdhC-H152R. Further cross-resistance testing will be carried out to fully characterise the impact of these single and double mutants on different SDHI fungicides.





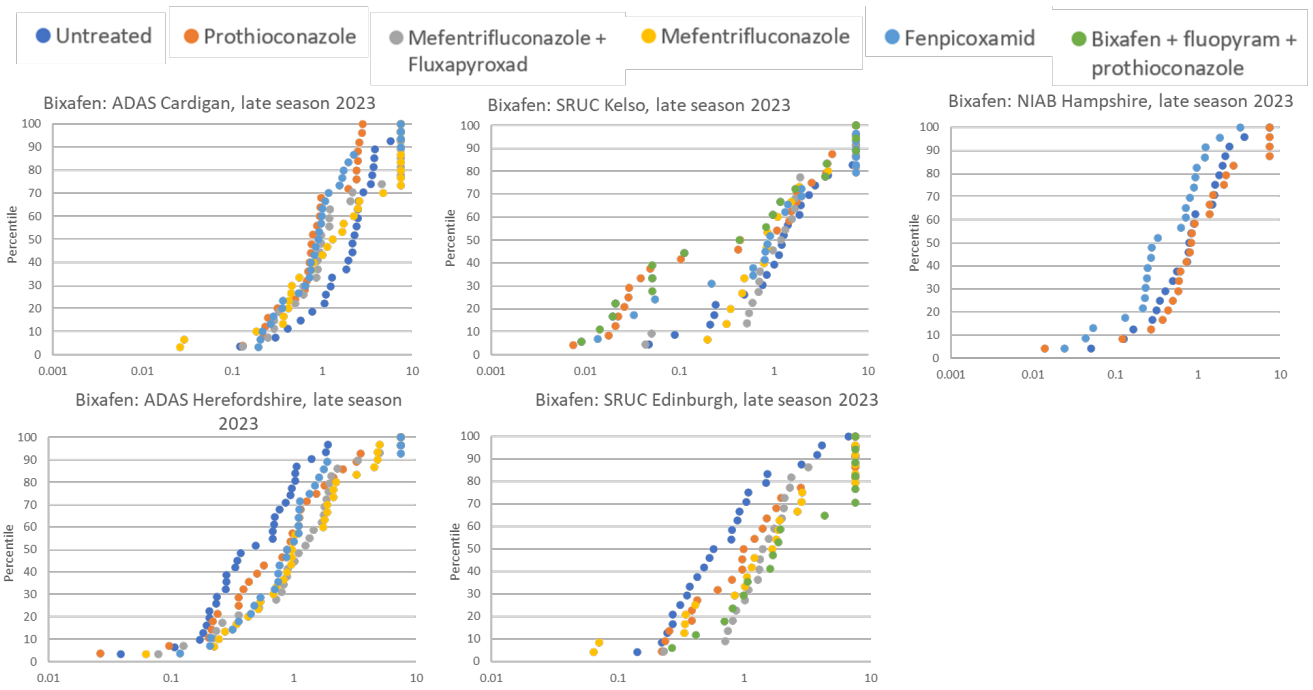
Sensitivity to a new SHA-SDHI fungicide was generally within a more similar range to 2022.

Annual Project Report
April 2023 to March 2024



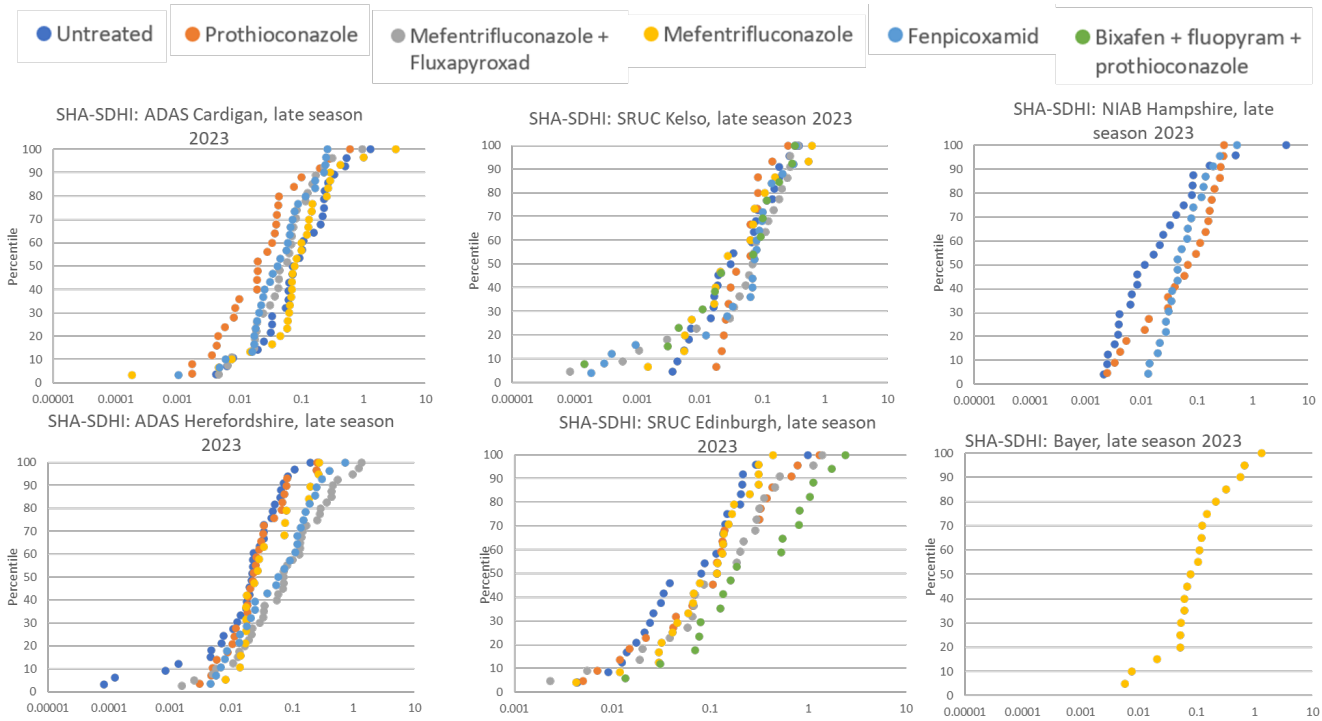
Late-season isolates also contained least sensitive isolates at frequencies >10% from multiple samples, but without a clear effect of SDHI-containing treatments within-season. In some sites, solo mefentrifluconazole-treated samples had similar bixafen resistance levels to SDHI-treated samples. For 2024, samples will be taken from isoflucypram-treated plots, to see whether stronger selection is exerted by a new active SDHI used a solo product rather than the SDHI-azole mixtures tested in 2022-2023. Sequencing of selected late-season isolates with a range of SDHI sensitivity levels is currently in progress.

Annual Project Report
April 2023 to March 2024



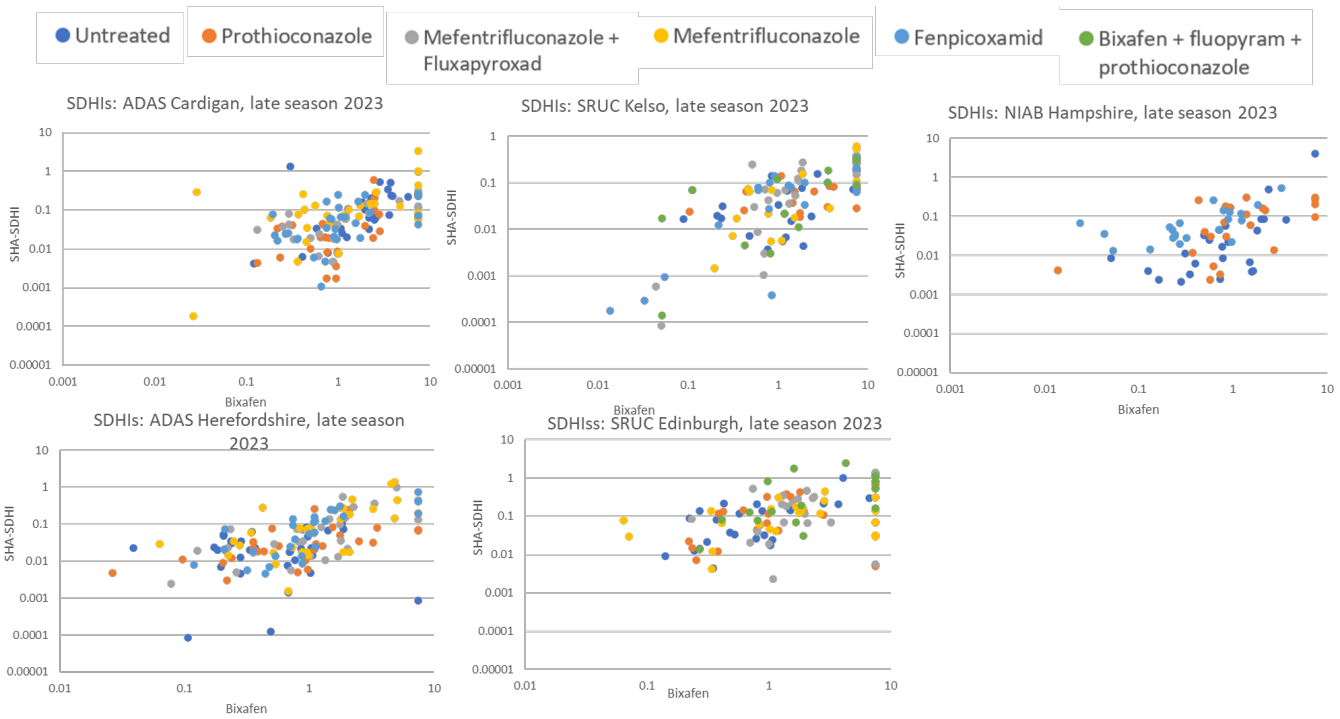
Sensitivity to the new SHA-SDHI in late-season samples showed little effect from the SDHI-containing treatments tested in 2022, except the bixafen + fluopyram + prothioconazole mixture from the SRUC Edinburgh site, which could be expected due to fluopyram treatment, but the same effect was not seen at the SRUC Kelso site.

Annual Project Report
April 2023 to March 2024



Partial positive cross-resistance can be seen between the two SDHIs, with the bixafen least sensitive isolates also mostly showing lower sensitivity to the SHA-SDHI. Further cross-resistance testing across a wider range of SDHI fungicides will be carried out.

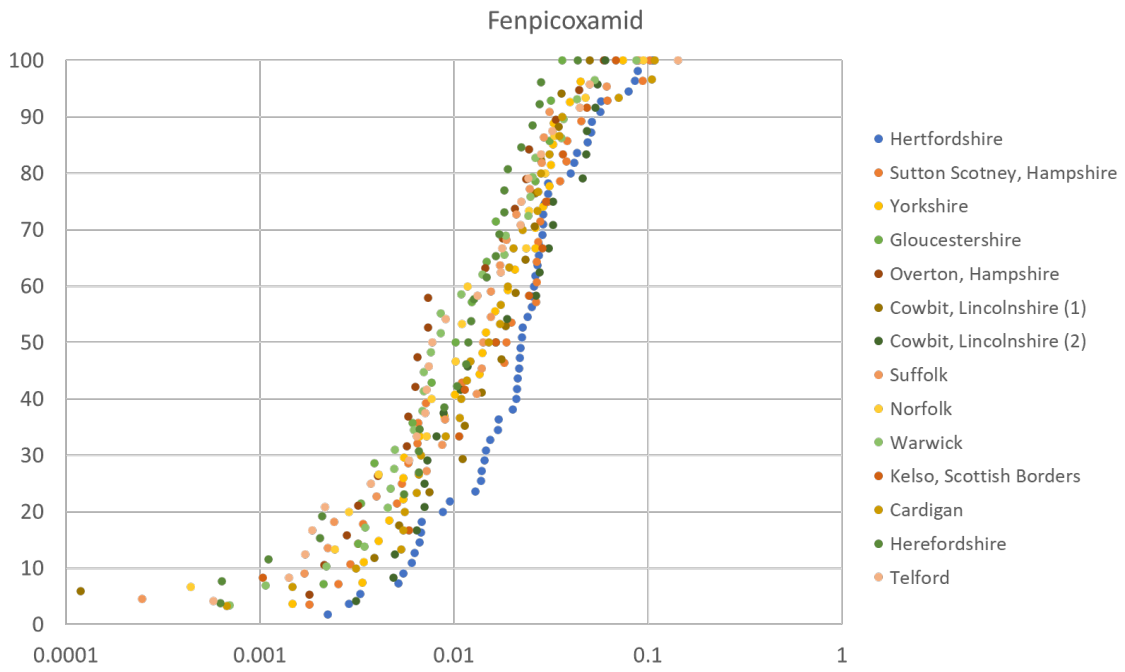
Annual Project Report
April 2023 to March 2024



Qil sensitivity

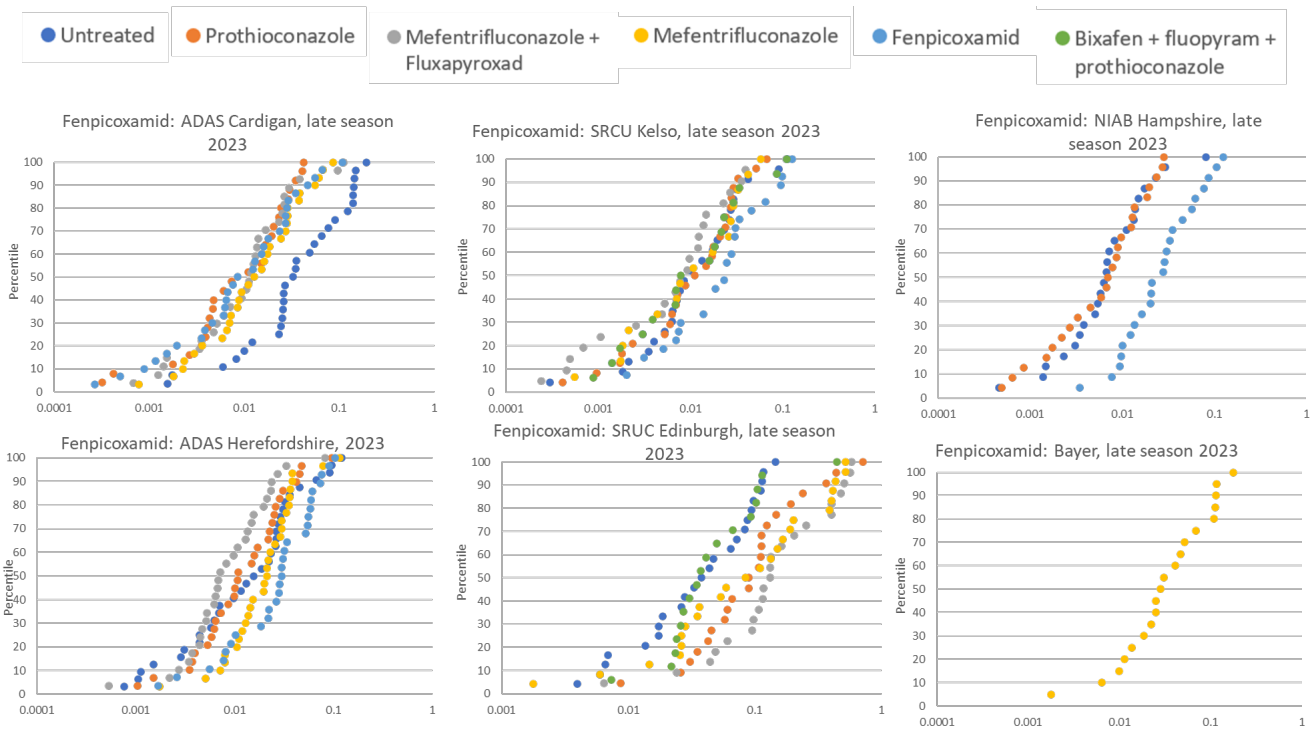
Early-season 2023 isolates had fenpicoxamid sensitivity within the previously established baseline range for all sampled sites.

Annual Project Report
April 2023 to March 2024



Late-season isolates also had sensitivity levels within the baseline range, but for three out of four fungicide performance sites where fenpicoxamid-treated plots were sampled, a small (<5-fold) reduction in population sensitivity was observed. This is a low-level shift and does not indicate target-site resistance, but these isolates will be further characterised to determine whether non-target-site mechanisms such as enhanced efflux or alternative oxidase activity are involved, and further field or glasshouse testing would be required to establish whether this has any impact *in planta*. Fenpicoxamid-treated plots will be sampled again in 2024, to identify whether this potential further selection is consistently seen.

Annual Project Report
April 2023 to March 2024



Key issues to be addressed in the next year

- Cross-resistance profiles of different sdh single/double mutants to different fungicides within the SDHI class
- Further shifts in frequency of SDHI least sensitive isolates at different sampling locations, and selection of sdh variants in isoflucypram-treated plots in 2024
- Sequencing of isolates with reduced sensitivity to prothioconazole and/or mefentrifluconazole, and cross-resistance of key CYP51 variants to different azoles
- Further shifts in azole sensitivity at different locations and prothioconazole or mefentrifluconazole-treated plots in 2024
- Non-target-site factors associated with QiI sensitivity variation within the baseline range
- Frequency and additional resistance factor contribution of MgMfs1 efflux overexpression in combination with different sdh and CYP51 target-site variants

Annual Project Report
April 2023 to March 2024

Lead partner	NIAB
Scientific partners	ADAS, SRUC and Teagasc (all unfunded)
Industry partners	BASF, Bayer CropScience, Corteva Agriscience, and Syngenta
Government sponsor	none

Has your project featured in any of the following in the last year?	
Events	***Press articles***
Cereals event 2023: this work formed part of a poster board describing fungicide resistance research at NIAB, alongside crop plots with different fungicide treatments.	
Conference presentations, papers or posters	***Scientific papers***
Results were presented alongside fungicide performance data at the AHDB Agronomy Conference (Dec 2023). Oral presentation were delivered at the International Congress of Plant Pathology, Lyon, August 2023 “The Evolution of Fungicide Resistance in European Cereal Pathogen Populations”, and at the Annual European Extension Group of Applied Plant Pathology Researchers meeting on 22 Feb 2024.	
Other	
Results presented at AHDB Fungicides Working Group meetings on 16 Oct 2023 and 2 Feb 2024, as well as FRAG-UK meetings on 30 Nov 23 and 20 Mar 2024. Results were also presented to regional agronomists at NIAB TAG Agronomists Conference on 9th Jan 2024 and farmers at NIAB East Professional Development Day on 8th Feb 2024.	