AHDB

from theory to field

The road to resistance?

> *Additional CYP51 mutations will evolve eventually that can coexist with others.*

With questions raised over fungicide resistance in OSR and the best course of preventative action, an AHDB project set out to discover the current status quo and look at the best tactics to protect fungicide efficacy in the field. *CPM* investigates.

By Lucy de la Pasture

While resistance management strategies have become a core part of fungicide programmes in cereal crops, oilseed rape has been somewhat lagging behind. A research project, funded by AHDB and partnered by industry, has been evaluating the risk of resistance developing in the main OSR diseases and looking at the best way to manage this risk in a cost-effective way.

Catherine Harries, who manages cereal and oilseeds disease research at AHDB, says there's a very real threat of fungicide resistance developing in OSR diseases. "Azole-insensitive strains of *Pyrenopeziza brassicae*, which causes light leaf spot (LLS), are already present in the UK. In Europe resistance to SDHIs has been reported in *Sclerotinia sclerotiorum* (sclerotinia) and further afield in Australia, azole insensitivity has been found in *Leptosphaeria maculans* (phoma stem canker). Such strains are likely to occur here eventually," she says.

Catherine says the aim of the project has been to pre-empt the development of fungicide resistance and put in place cost-effective strategies to help maintain the efficacy of fungicides.

Single-site chemistry

"In OSR there's a dependence on single-site modes of action which aren't generally applied in mixture with fungicides from other groups. From our experience in cereal crops, we already know this isn't a good resistance management strategy."

Charged with managing the project is plant pathologist Dr Faye Ritchie, based at ADAS Boxworth in Cambridgeshire. She explains when the project started in 2016, growers were having to very much rely on azole chemistry to control both phoma and LLS in the autumn. The situation became worse following the loss of Refinzar (penthiopyrad+ picoxystrobin) in 2018, which had provided an autumn alternative to azole chemistry in the form of an SDHI and strobilurin (QoI) co-form.

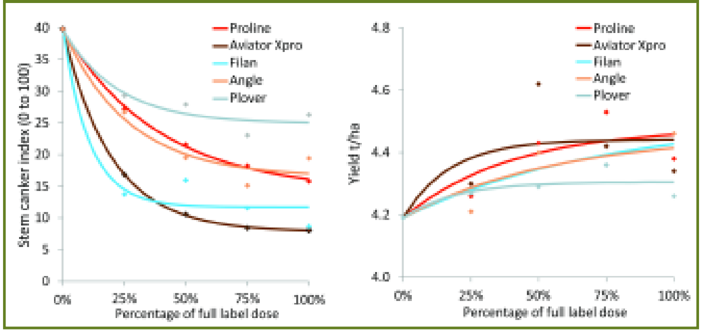
"The first stage of the project was to look at the risk of fungicide resistance developing in the pathogens causing LLS, phoma stem canker, sclerotinia and alternaria (*Alternaria brassicae/brassicicola*). We did this using a published peer-reviewed model to predict how long it was likely to take for resistance to occur for all the modes of action and active ingredients available to control OSR diseases," explains Faye.

"The results showed the risk of resistance developing is very similar across all modes of action. The different pathogens showed more variation, with sclerotinia having the lowest risk of developing resistance and alternaria the highest, though very closely followed by LLS and phoma."

Having established the risk of fungicide resistance developing, Faye explains that LLS was the main concern. "We know the mutations that cause azole insensitivity are already present in the UK pathogen population. In 2015 the LLS epidemic was very difficult to control in OSR crops, with multiple fungicide applications being commonplace as growers struggled to

Faye Ritchie reports the LLS mutation resulting in G460S is dominant and present in 70-90% of the population in 2017-18, substantially higher than in 2011.

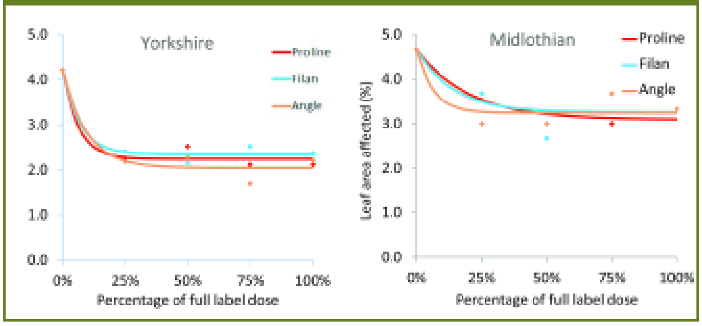
Phoma stem canker



Four trials at Rosemaund, Herefordshire and Terrington, Norfolk.

Source: AHDB Fungicide Performance trials, 2018-2019.

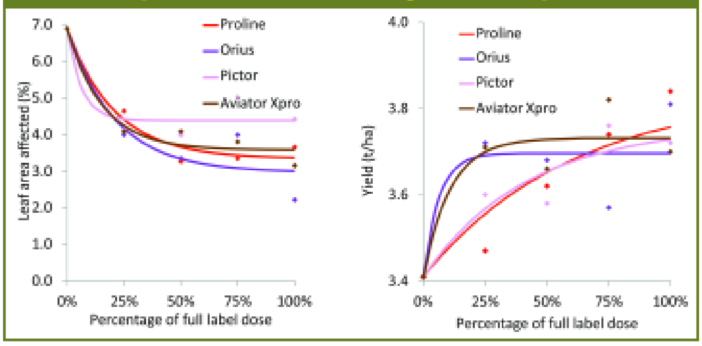
Light leaf spot



Disease levels assessed in March 2019. Note - labels for Filan and Angle do not include control of light leaf spot.

Source: AHDB Fungicide Performance trials, 2018-2019.

Aviator performance on light leaf spot



Five trials in Yorkshire and Midlothian.

Source: AHDB Fungicide Performance trials, 2015-2016.

control it and we needed to understand why," she says.

LLS is a polycyclic disease which makes it difficult to control because the pathogen is always present in the crop at different stages of its lifecycle. "Looking at historic data, LLS control from fungicides ranges from 40-85% in AHDB's fungicide performance trials, with the best efficacy coming from an autumn fungicide followed by a further application in the spring," highlights Faye.

During the three years of the project, samples of LLS were sent from field trials to Rothamsted Research to test for the presence and identity of mutations associated with decreased sensitivity to azoles and to assess their prevalence in the population.

Researchers at Rothamsted had previously identified two alterations in the LLS CYP51 protein, the target site in azole chemistry, known to be associated with decreased azole sensitivity — G460S and S508T. They reported the mutation resulting in G460S to be dominant and present in 70-90% of the population in 2017-18, which was substantially higher than in earlier testing in 2011 where it was only found in approximately 20% of isolates, explains Faye.

In addition, the majority of strains are now also able to overexpress CYP51 after exposure to azoles due to the presence of different DNA inserts in the promoter region of the encoding target gene.

"The unexpectedly high levels of CYP51 promoter inserts in combination with G460S in the population at the start of the season made it very difficult to compare resistance management strategies by testing for selection, and analysis is ongoing to determine the conclusions we can draw from the work."

Still effective

In spite of the high proportion of the LLS pathogen carrying this mutation, azoles are still effective in controlling the disease in the field. "Even though we found this mutation to be widespread in all eight trials, there was no difference in the performance of azoles and non-azoles between sites."

This has been further backed up by the AHDB fungicide performance trials, she highlights, where no further shifts in azole performance have been detected. That begs the question, why is this?

Faye explains that the significant decline in azole performance against septoria in wheat has been mainly associated with the accumulation of multiple mutations in the CYP51 encoding gene of the septoria pathogen.

"In lab studies at Rothamsted, if the two known LLS mutations occur together in the CYP51 protein then azole sensitivity can be up to 35 times less in yeast transformants. But it's thought that both mutations can't evolve together in nature due to fitness costs, which may be why azole

Of all the OSR pathogens, LLS is causing most concern because azole-insensitive strains are already present in the UK.

field performance is currently not severely affected.

"But it's possible that additional CYP51 mutations will evolve eventually that can coexist with others and lead to a significant decrease in azole performance. We don't know what the next step will be in the evolution of the pathogen or when it will happen, which is why we need to have a resistance management strategy already in place," explains Faye.

There's also still more to be understood about the contribution of CYP51 overexpression and mutations to azole insensitivity and levels of LLS control, she adds.

The project assessed the use of azoles alone against azole/non-azole tank mixtures and alternating azoles with different modes of actions, as the latter two are recommended strategies for resistance management. When it comes to deploying these strategies in the field, Faye emphasises it's important to consider fungicide use over the whole growing season, rather than consider treatments for specific diseases in isolation.

"Pathogens can be present across the entire growing season, even if they are not the target of a fungicide application. It's the length of exposure to a particular mode of action that creates selection pressure, not the actual amount of disease present. As an example, there's likely to be some LLS present at low levels when a fungicide is applied for sclerotinia control during flowering, but ▸

Modelling work shows sclerotinia is at the lowest risk of developing resistance and alternaria the highest, though very closely followed by LLS and phoma.

Research roundup

AHDB Project No 21120015: 'Maximising the effective life of fungicides to control oilseed rape diseases through improved resistance management' is led by ADAS in partnership with Rothamsted Research, Adm, BASF, Bayer, Corteva and Syngenta. It runs from Jan 2017 to June 2021, at a cost of £160,966.

AHDB Project No 21120013: 'Fungicide performance in wheat, barley and oilseed rape' is led by ADAS in partnership with Harper Adams University, National Institute of Agricultural Botany and Scottish Rural University College. It runs from June 2018 to March 2022, at a cost of £732,234.
ahdb.org.uk/fungicide-performance

From Theory to Field is part of AHDB's delivery of knowledge exchange on grower-funded research projects. *CPM* would like to thank AHDB for its support and in providing privileged access to staff and others involved in helping put these articles together.

▸ often it isn't the target."

In recent seasons finding an active ingredient that wasn't an azole to use in the autumn has been nigh on impossible, but two new products gained approval in 2019, adding co-forms containing additional modes of action for phoma and LLS spot control.

Aviator Xpro (bixafen+ prothioconazole) has been approved for control of light leaf spot, phoma stem canker and sclerotinia. Independent assessment in AHDB's fungicide performance trials show it tracks the performance of straight prothioconazole very closely for LLS control, though in terms of yield the trials show an advantage over prothioconazole alone.

Angle (azoxystobin+ difenoconaole) has an approval for phoma stem canker reduction and sclerotinia control (moderate), though in the fungicide performance trials also showed control of LLS.

Faye highlights that when using co-forms as part of a resistance management strategy it's useful to know the relative strengths of its active ingredients on the different diseases. "For example, if one part is strong on a disease and the other has no activity against the same disease, then relying on this co-formulation as a mixture isn't a good resistance management strategy."

Low disease pressure

Faye points out the field trials took place during three low pressure disease years. "According to the Defra oilseed rape disease survey statistics, in 2016-2018 only 12-15% of OSR crops were affected by LLS. This compares with 23-44% in the period 2008-2016," she explains.

"We found that, where the same fungicide programmes were compared across three years and eight sites, gross margins were not significantly different from where no fungicides had been applied," she says.

"A yield response of 0.17-0.27t/ha was necessary to cover the cost of fungicides and associated yield responses achieved in trials just covered the cost, despite low disease pressure. From the industry sponsored trials, a range of different fungicide products/alternation and tank mixture strategies were tested.

"It was found that mixtures were generally the most expensive strategy, followed by alternation with the azole-only strategies coming out as being the least expensive. The conclusion from this is that having access to good information on the efficacy of products, at a range of doses, will be key to putting together a cost-effective resistance management fungicide programme.

"Resistance management strategies don't have to be more expensive than azole-only fungicide programmes, and by implementing them it will make sure that the efficacy of active ingredients is preserved for longer," she concludes. ■

A start to seeing the bigger picture

While fungicide resistance management strategies have been well developed in cereals, in other crops it's an area where research has been lacking, says BASF's Clare Tucker.

"In oilseed rape, there's been a real dependence on azoles, particularly early in the programme, but also a lack of research into how fungicide use may affect the development of resistance in pathogens. The LLS pathogen is present in the crop practically from the get-go and phoma infects in early autumn, so these pathogens are exposed to fungicides over a long period of time. In comparison, fungicide applications only start in the spring for cereals.

"Getting a feel for what's going on in the OSR pathogen population will help make informed decisions and identify where more work needs to be done. That's what makes this project so valuable — it's good fundamental science that's been a long time coming," she says.

Even though the economics of applying resistance management to fungicide strategies didn't always fall the right side of positive in this trial series, Clare points out that fungicides have to be applied in a preventative manner when there's no way of knowing how the disease will develop over the season.

"Because OSR is also a very elastic crop, yield is not just determined by good disease control. It's very different to wheat, where there's a very direct relationship between levels of foliar disease and yield. In OSR, there's a much more complex relationship with factors such as cultivar interactions, canopy size and canopy duration all influencing yield. It's a jigsaw that needs to be put together to optimise yield by focusing inputs on a more individual crop basis," she says.

Clare believes the project provides a good platform for further research to look a bit more closely at how this puzzle comes together. "As a manufacturer, we strongly support the agronomy work ADAS carry out looking at the components of yield and the outcomes of this project will help build on this important agronomy work."

Clare Tucker says OSR pathogens are exposed to fungicides over a longer period of time than cereal crop pathogens.