Too keen to be clean?

HGCA

from theory to field

Grower practice will drive how fungicide resistance develops, suggests the latest research. *CPM* analyses the facts to gain a better understanding of how the situation may evolve and seeks clear guidance on how to slow down its effect in the field.

By Tom Allen-Stevens

Take a look at the wheat in this picture — it's perfect, you may say. And if you look closely, you'll probably not find a speck of disease from its fulsome ear to the base of its sturdy stem. This is just the sort of polished crop you may be aiming to have in your fields in a few months' time, and you may be planning to make successive visits with the sprayer to achieve it.

Well if you are, you could be building up a problem, not just for yourself, but for your neighbours, too. On-going research into fungicide application practice and its effect on septoria populations is revealing some startling new truths about how some aspects of the drive to keep the crop clean are creating a pathogen that's more difficult to control, says Dr Paul Gosling of HGCA.

"What's now becoming clear is that the treatment decisions growers make in the field have a direct effect on how resistance to fungicides evolves. If you manage a crop so that it's clean to the ground, it doesn't necessarily mean you've taken out the strains of septoria that are hardest to control. You've more likely removed the easy ones, and the tough septoria will multiply and come back in subsequent years."

Driving resistance

Indeed, growers using azoles right from the T0 spray timing through to T3 could be driving resistance harder and harder, according to preliminary results of one study, he points out (project 3800 — see panel on p21). "Initial findings from that project are based on just one year's results. But it's confirming findings from previous studies by Dr Frank van den Bosch at Rothamsted Research. Where growers are going on with an azole-based T1.5 or T4, this is making matters worse."

The next step will be another year of field trials to validate the findings so far, and to refine the model of how resistance develops. There'll also be a greater focus on the difference varietal susceptibility can make, he notes.

Running alongside the project was a major industry-funded study, focused on establishing a framework for sustainable use of SDHI fungicides. This project shaped guidelines issued by the Fungicide Resistance Action Group (FRAG), released last spring:

- Maximise the use of multi-site chemistry throughout the spray programme
- Limit the number of azole treatments
- Use robust azole doses at the T1 and T2 timings when mixing with SDHIs
- Use the minimum number of SDHI treatments and dose required to achieve effective disease control.

"Growers now have clear guidance, backed up with evidence from independent scientific trials, on how best to use SDHIs to preserve their value for the long term, as well ▸

"With the combination of pesticide regulation and fungicide resistance, we could be heading for a potential car crash."

▸ as how azoles should best be managed," points out Paul Gosling.

Building into this research is the analysis of septoria isolates taken from the fungicide efficacy trials, he continues (project 3713 — see panel on p21). "One worrying development is the emergence of an enhanced efflux mechanism in field populations, where septoria isolates have acquired the ability to pump a fungicide out of their cells — we need to know more about how this plays out in the field, with regard to fitness."

Intense fungicide use

The number of fungicide applications made to crops is another concern, notes Dr Neil Paveley of ADAS, who leads the current project studying intensive fungicide use. "Since the early 1990s, the Defra cereal disease survey shows that the average number of treatments has risen from fewer than two to around 3.5 applications. Yet it's difficult to argue that we're achieving substantially better control of the disease," he points out.

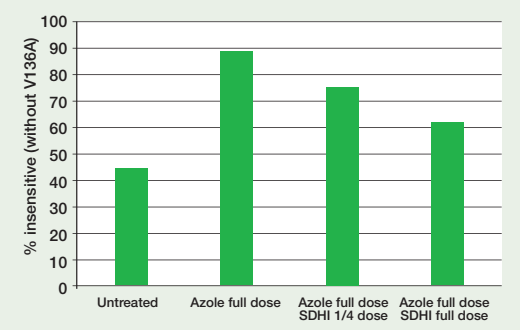
"So with the combination of pesticide regulation removing active substances from use and fungicide resistance affecting the efficacy of those that remain, we could be heading for a potential car crash. We need to take avoiding action, particularly because the next new mode of action is several years away."

So what's driving the increase in the number of applications? The leading fungicides are as effective, if not better, despite the drop-off in azole activity on septoria, argues Neil Paveley. "As an example, If you compare the performance today of a half dose of Seguris (epoxiconazole+ isopyrazam) with that of Tilt (propiconazole) 20 years ago, the spray window is at least as wide, and you get better control at the peak."

Yellow rust has also changed — the aggressiveness of the Warrior race has shortened the period between infection and sporulation by around two days, he notes. Moreover, there's now an increased focus on mycotoxin control. "So there are perfectly legitimate reasons why fungicide use has increased at either end of the spraying season."

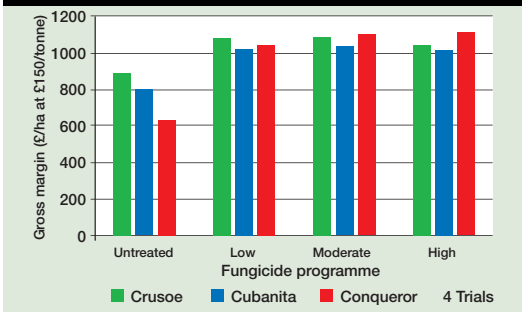
Added to this, there's the septoria

Effect of mixing fungicides on sensitivity of septoria



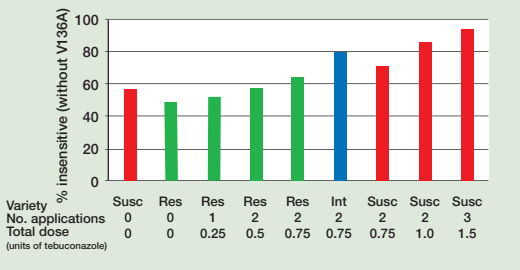
Adding an SDHI to tebuconazole resulted in fewer insensitive strains (those without CYP51 V136A), but that then exposes the SDHI.

Response of variety to fungicide programmes



Source: ADAS, 2014

Selection for azole-insensitive septoria



Source: ADAS Boxworth, 2014, sample taken at GS65

Fungicide programmes under test

	Spray timing	T0	T1	T2	T3
Intensity of programme	Nil	Untreated	Untreated	Untreated	Untreated
	Low	-	Azole+ strob+ multi-site	Azole+ multi-site	
	Moderate	Mildewicide+ multi-site	Azole+ strob+ multi-site	SDHI+ azole+ multi-site	Azole
	High	Azole+ mildewicide+ multi-site	SDHI+ azole+ strob+ multi-site	SDHI+ azole+	Azole multi-site

population itself. Since 2010, samples drawn from fungicide efficacy trials have been tested for resistance at a molecular level and analysed for the impact they have on fungicide efficacy. There are now around 70 different variants of the CYP51 protein targeted by azoles, based on combinations of mutations. Generally, where more mutations have stacked in individual septoria isolates, this has conferred the pathogen with a greater level of resistance, and growers have responded by increasing the dose and frequency of treatment to maintain control.

Two recent discoveries have been overexpressing strains, where isolates can produce more of the CYP51 protein and thereby reduce sensitivity to azoles, and a mechanism known as enhanced efflux. "This is where the fungus pumps more azole out of its cells due to overexpression," explains Dr Bart Fraaije of the Fungicide Research Group at Rothamsted Research.

"We've been picking up isolates at low frequencies (0-6%) in UK populations showing reduced sensitivity to both azoles and SDHIs since 2013 — it's another step in the arms race."

Analysis has confirmed these isolates have the efflux mechanism and tests in a petri dish have shown they need around 5-10 times as much fungicide — including azoles and SDHIs but not multi-sites — to control them, compared with an isolate that doesn't have these mechanisms.

"This all sounds worrying, but it needs to be put into context," continues Bart Fraaije. "That change in sensitivity is relatively low in comparison with most CYP51 mutations. Because they still occur at a very low frequency, these strains are also likely to be unfit. So in the immediate future, they'll make very little difference to practical control in the field.

"But it's a very strong warning shot across the bows that this pathogen is continuing to evolve, and will overcome the SDHIs in due course, most likely due to SDHI target-site ▸

Treatment decisions growers make in the field have a direct effect on how resistance to fungicides evolves, says Paul Gosling.

The average number of fungicide treatments a cereal crop receives has risen from fewer than two to around 3.5 applications, notes Neil Paveley.

▸ changes," he cautions. "The questions that remain are how quickly it will achieve these, and what can be done to slow up that process."

One outcome of the work that's helping the research progress is the development of an in-field diagnostic test for resistance. A septoria lesion can be analysed to determine whether there are CYP51 and/or efflux pump overexpressing stains present.

"This still needs further evaluation before it works reliably, but should be ready by Cereals this year. So we'll be able to use it in the field this autumn to find out more about the threats from septoria growers will be facing in spring 2016. It's more of a research tool, enabling rapid monitoring, than of direct use to growers, though," reports Bart Fraaije.

Meanwhile, the effect on the septoria population of varying SDHI and azole dose on selection of mutations conferring resistance has been tested in the field, using insensitivity against tebuconazole as an experimental system to understand how azole resistance evolves. The proportion of the population that was insensitive to tebuconazole was measured after application of either tebuconazole alone or in mixture with an SDHI (see chart on p18).

Selection for resistance

"A full dose of tebuconazole essentially removes nearly all the sensitive isolates and replaces them with insensitive ones. If you add SDHI, selection for azole resistance is reduced, so you get a good mixture effect. We use tebuconazole as it is a simple system to monitor, although the same principle applies to other azoles. But the problem is, that then exposes the SDHI," notes Bart Fraaije.

So how do you effectively balance a programme so that it puts the minimum amount of pressure on the chemistry, but still delivers adequate disease control? Two essential tools in the armoury are multi-site fungicides and varietal resistance, notes Neil Paveley.

"You can think of variety disease resistance as the equivalent of adding another mode of action to a fungicide mix," he notes. "The difficulty is there tends to be a yield penalty with disease-resistant varieties."

Both multi-site fungicides and varieties are under investigation in the new study (project 3800), that has now completed its first year of field trials. "There are two strands of work — we're looking at mixtures and modes of action to determine the best way to slow down resistance. Then we're also using disease-resistant varieties and getting evidence of their benefit," he explains.

Some septoria strains have evolved a mechanism known as enhanced efflux where the fungus pumps more azole out of its cells due to overexpression.

There are four different treatment regimes (see table on p19), ranging from nil to four azole doses applied and from nil to two SDHIs. These are tested on Crusoe, with an HGCA Recommended List rating of 6 for *Septoria tritici,* Cubanita (5) and Conqueror (4).

"As the intensity of the programme goes up, so too does the yield, and in 2014 we had a 4.5t/ha yield response in Conqueror," reports Neil Paveley. "But if you then look at

Knowledge on septoria informs trials programme

For Mike Thompson, coordinating the AICC trials, the HGCA-funded fungicide research is opening a fascinating window on the key pathogen growers have to tackle. "I think it's amazing what we now know about the mechanism of resistance in septoria — it's a definite step forward in informing us how to put together fungicide programmes."

It's these programmes that are now coming under scrutiny. "What's interesting is that the response from growers to the high disease pressure they've faced has been to increase the azole intensity. But that will just speed up the train crash.

"So we should be considering alternative approaches that maintain the level of control, but rely less on azole chemistry. It's one area that we're looking at within the AICC trials programme."

Where yellow rust, rather than septoria is the issue at T0, that's an opportunity to switch to alternative modes of action, for example. "If sticking with an azole at T0, one option I favour is to use a different azole to the one you'd use at T1 and T2."

This year, he'll use epoxiconazole or prothioconazole at the main T1 and T2 timings. "For early sown or high-yielding varieties, I'd certainly consider an SDHI at T1, as well as at the T2 timing. But I'd make sure the rate of azole was at least a three-quarter dose, to give adequate protection to the SDHI."

But the key to achieving both good disease control and preserving the chemistry is to get the timing right, reckons Mike Thompson. "We're losing the kick-back activity of the azoles, so it's becoming even more important to time them right — if we rely on SDHIs for this activity, we'll be exposing them dangerously to resistance issues.

"Getting the timing right will also help reduce the number of azoles needed in the programme. But there's rarely a need to apply a pre-T0 azole, and if a T1.5 is needed, perhaps that should just be a multi-site," he suggests.

Mike Thompson has been looking at alternative approaches that maintain the level of control, but rely less on azole chemistry within the AICC trials programme.

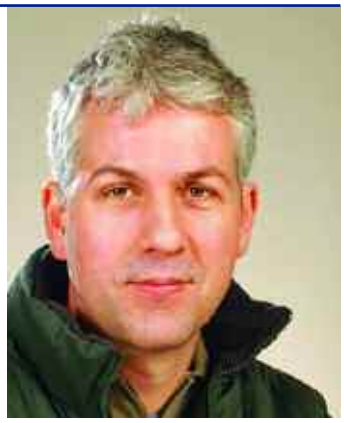
Research round-up

HGCA project 3800, Consequences of intensive fungicide use or integrated disease management for fungicide resistance and sustainable control, runs from Jan 2013 to Sept 2016. Its aim is to quantify the effect of integrated disease management on selection of fungicide-resistant pathogen strains, translate this into implications for the effective life of fungicides, and interpret the implications. Its total cost is £691,546, and the project has industry partners Adama, BASF, Bayer CropScience, CRD, Defra, Dupont, Limagrain and Syngenta. HGCA funding amounts to £40,000, with the research carried out by ADAS and Rothamsted Research.

HGCA project 3517, Improved tools to rationalise and support stewardship programmes for SDHI fungicides to control cereal diseases in the UK, ran from Jan 2010 to Jan 2014. Its aim is to provide a scientific framework for sustainable use of SDHI fungicides and their mixing partners to maintain or enhance cereal production in the UK. Its total cost was £1,221,386, funded through the Defra-sponsored Arable LINK programme with industry partners BASF, BayerCropScience, Dupont and Syngenta. HGCA funding amounts to £119,993, with the research carried out by Rothamsted Research, ADAS, SRUC and Velcourt.

HGCA project 3713, Identification and characterisation of azole sensitivity shifts in Irish and UK populations of Mycosphaerella graminicola sampled from HGCA-Fungicide Performance winter wheat trials, ran from April 2011 to March 2014, and has now been extended to April 2019. Its aim was to identify changes to the CYP51 gene targeted by azoles in field populations of septoria leaf blotch, to link these to field applications of fungicide treatments, and to establish if alternative resistance mechanisms are evolving. Its initial cost was £60,000 funded by HGCA. The research was led by Rothamsted Research, with partners ADAS, NIAB TAG, SRUC and Teagasc.

● For more information, go to *www.hgca.com*



Bart Fraaije has been picking up septoria isolates in UK populations showing reduced sensitivity to both azoles and SDHIs since 2013.

the economics of it and consider gross margin, the whole thing evens up." (See chart on p18).

What the researchers found was that the resistant variety on a low intensity programme returned almost as much as a susceptible variety under a high input regime. "This was only one year's results, but I was amazed the low-intensity programme achieved anything like the margins it did in a high-disease year like 2014," remarks Neil Paveley.

The effect on the septoria population was clear, he continues, albeit from just one year's results (see chart on p18). "As you build the number of azole applications on a susceptible variety, you can see just how quickly you drive resistance harder and harder. Whereas fewer treatments on a more septoria-resistant variety slows up fungicide resistance."

The trials are the first to have provided such good evidence of this effect, he notes, as well as figures to quantify it that can be built into modelling work. "If this is happening to tebuconazole-insensitive strains, it will also be happening to the new, worrying strains that are harder to control, but currently only make up about 1% of the population," he warns.

So when building a spray programme, multi-sites, which are at low risk of fungicide resistance, should be used throughout to reduce the risk of selection.

"Limiting the number of azole applications will be a challenge, but where they are used at T1 and T2, the dose should be robust, either to protect the SDHI or, if used with an alternative partner, to achieve good control. With SDHIs, both dose and frequency of application should be the minimum needed to achieve good control."

While multi-sites should be used for septoria protection, strobilurins and morpholines should be considered where rusts are the priority. "Growers should also consider that varietal resistance can offer an extra mode of action, and it's encouraging to see there's already a shift towards growing more resistant types."

But most of all, he advises against heading down the same route as potatoes and increasing the number of fungicide applications. "The potato blight pathogen is mainly asexual and unusually poor at developing resistance, whereas septoria reproduces sexually and is really very accomplished at overcoming fungicides.

"If you put the pathogen under intense fungicide pressure, it'll just be the hard cases that survive and have sex with each other, and their offspring come back next year. So we need to rethink what successful disease control looks like in the field and how we achieve it." ■

The trials have shown how quickly you drive resistance harder and harder if you increase the number of azole applications on a susceptible variety.