Behind the lines of resistance

> *"Septoria is a pathogen that will continually seek to out-manoeuvre growers."*

Technical Septoria management

For years, growers have been on the backfoot with septoria control, fighting a pathogen that's grown in strength as its population has evolved. *CPM* gathers the intelligence to give growers a credible advantage against their foe.

By Tom Allen-Stevens

In 2016, AHDB fungicide performance monitoring began to pick up a worrying change. The flagship products growers and agronomists rely on for managing *Septoria tritici* were showing a notable drop-off in performance.

Ever since the new generation SDHIs had been introduced there had been warnings that resistance was inevitable, and the monitoring work had already tracked the decline of azole efficacy. But no one was sure how soon the performance of newer chemistry would be affected, nor how quickly it would tumble.

"There was a distinct shift in sensitivity in the septoria population, reflected in the performance of the main SDHI/azole fungicides over two years," notes Jonathan Blake of ADAS. "Over the past two years, however, field efficacy within the fungicide performance trials appears to be fluctuating.

"What's unclear is how much we can rely on the current chemistry we have — how robust are the genetics in the wheat varieties currently grown, and how is the septoria population shifting? And with the loss of chlorothalonil, will the selection pressure intensify?"

Jonathan notes something of an "anomaly" in the 2020 performance curves that suggests Ascra Xpro (bixafen+ fluopyram+ prothioconazole) has recovered some of its lost efficacy. "But Revystar XE (fluxapyroxad+ mefentrifluconazole) does look promising — it's turned back the clock in terms of efficacy. It'll be interesting to see how it develops."

Higher performance

What concerns him, though, is the effect a series of low disease years will have had on perceptions of fungicide strength and efficacy. "The data suggest the protectant activity of prothioconazole has plateaued at about 40%. Revysol is showing a much higher level of performance, but growers could currently be putting less of an insurance margin into fungicide programmes without realising it. The background population is more resistant than it was the last time we had a bad year for septoria. With the loss of the most active multisite, chlorothalonil (CTL), we're also more reliant on just two modes of action, offered by the SDHIs and the azoles."

So just what is the current picture on resistance for these two key groups? Dr Rosie Bryson is head of technical project management for BASF across Europe. Every year, the company collects septoria isolates from across the continent as well as from the UK and Ireland and tests them to assess how the population is shifting in its sensitivity to the chemistry, she explains. The data and the understanding of complementary independent work and findings are shared and discussed between European scientists to agree a clear picture of just how the pathogen is developing.

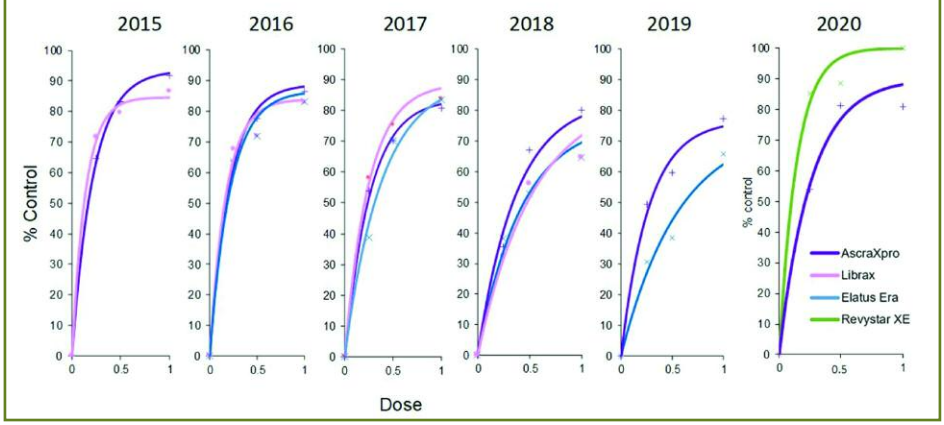
Rosie's brought together the data to help explain recent shifts in the efficacy of SDHIs. "Strobilurin fungicides went from hero to zero in just over a year in the early 2000s in the UK. For azoles it's been a much more gradual shift. With SDHIs, there's an erosion of efficacy, but it's nowhere near field failure at the moment," she says.

Adaptation — whereby an isolate mutates to one that is less sensitive to SDHIs — was first noted in net blotch and septoria in 2012. The progression of mutation development in net blotch was faster than in septoria but by 2016, mutations in septoria had also started to increase significantly in the UK and Irish population.

"Fairly early on, one mutation — C-H152R — was identified with a relatively high ED_{50},"

Jonathan Blake in unclear on how much growers can rely on the current chemistry and wheat genetics to keep deliver an effective defence against septoria.

SDHI + azole protectant activity over time



Source: AHDB, 2020; Librax contains fluxapyroxad+ metconazole; Elatus Era – benzovindiflupyr+ prothioconazole.

Rosie explains. This is the dose needed in lab tests (in vitro) to kill half of the isolates in a particular sample — the higher the ED_{50}, the more resistant the isolate.

"C-H152R was found to have a high level of insensitivity to all of the SDHIs, and this was at first alarming. But it currently accounts for a small percentage of the population in the field and we believe there's a fitness penalty meaning it's adapted to SDHI chemistry, but doesn't survive the winter well."

The UK and Ireland have a more resistant population than mainland Europe as a result of the generally wetter weather, higher resulting incidence of septoria and more frequent sprays applied. Two mutations dominate the population — C-T79N and C-N86S make up around 68% of isolates, with C-H152R accounting for about 4%.

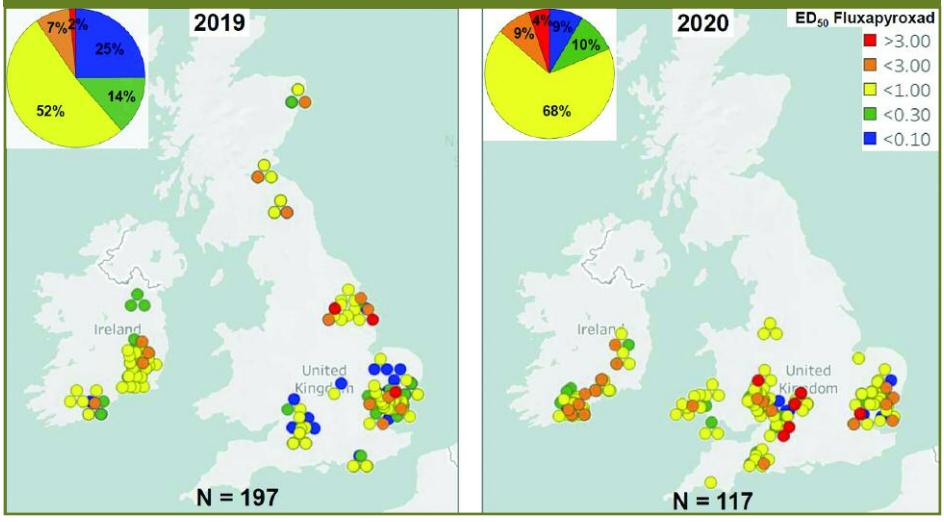
"The two dominant isolates have an impact, and efficacy of SDHIs varies — BASF tests have shown Xemium (fluxapyroxad) is not significantly affected, while the impact on other SDHIs depends on their intrinsic efficacy" adds Rosie.

What's interesting is how the changes in sensitivity of the septoria population have developed over time. This is usually illustrated through frequency distribution curves arranged according to ED_{50} value. "For azole-related mutations you see a gradual shift to the right over time. With SDHIs, the shift has been quicker, but you get a bimodal distribution curve," she explains.

"The population has shifted to one that's more resistant, but the key point is that it hasn't become more severe. If that had happened, you'd expect to see a third 'hump' in the distribution curve with high ED_{50} values. So we're not seeing a killer mutation, just an increase in frequency."

Rosie notes that the resistance mechanism generally found to SDHIs is target site meaning mutations at the fungicide target reduce the sensitivity of the isolate to the SDHI molecule. "All commercial SDHIs react in a similar manner and as such are cross resistant. But in 2016, Dr Bart Fraaije at Rothamsted Research discovered something rather odd during monitoring work he was carrying out. He found two wild type isolates with no SDHI mutations that showed a high degree of resistance to fluopyram."

ED_{50} of *Zymoseptoria tritici* towards Xemium – UK and Ireland



Source: BASF internal data, 2020.

With SDHIs, there's an erosion in efficacy, says Rosie Bryson but it's not field failure.

Strong cross-resistance was confirmed between fluopyram and isofetamid, but importantly not with other SDHIs. The conclusion reached was that this is a resistance mechanism outside of the target site of conventional SDHIs. "We therefore have two different mechanisms of resistance at play for SDHIs in the field, but target site resistance is by far the dominant one. That means using two SDHIs together is not an effective resistance management strategy for septoria," notes Rosie.

A small number of double mutations in a single isolate have also been identified, which have the effect of raising ED_{50} values. "We're monitoring these developments, but again incidence is currently very low. So current best practice of using an SDHI at no more than two timings, always mixed with at least one other mode of action, is still the best advice to retain the efficacy of this valuable chemistry," she advises.

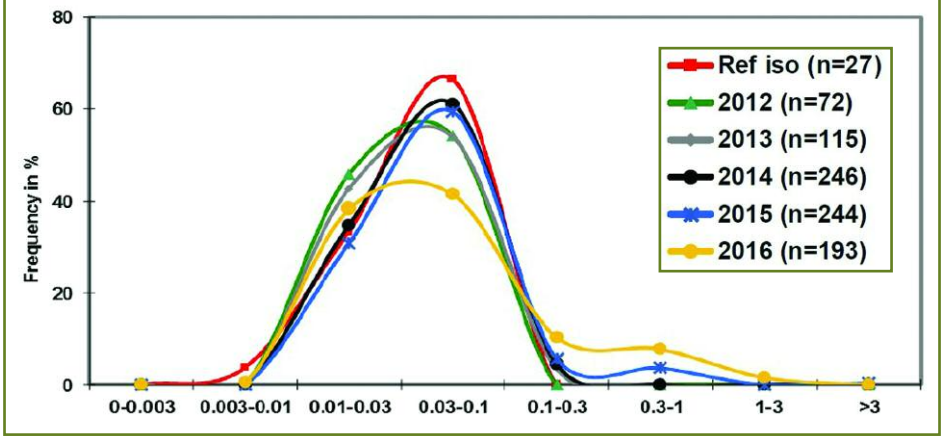
So what about the azole element of Revystar XE? Similar tests have been carried out across Europe to assess the sensitivity of septoria isolates to Revysol notes Dieter Strobel, responsible for technical market development of cereal fungicides.

"During product development, we had already established Revysol has an extremely high level of efficacy against septoria — even on those isolates that were less sensitive to prothioconazole (PTZ)," he notes.

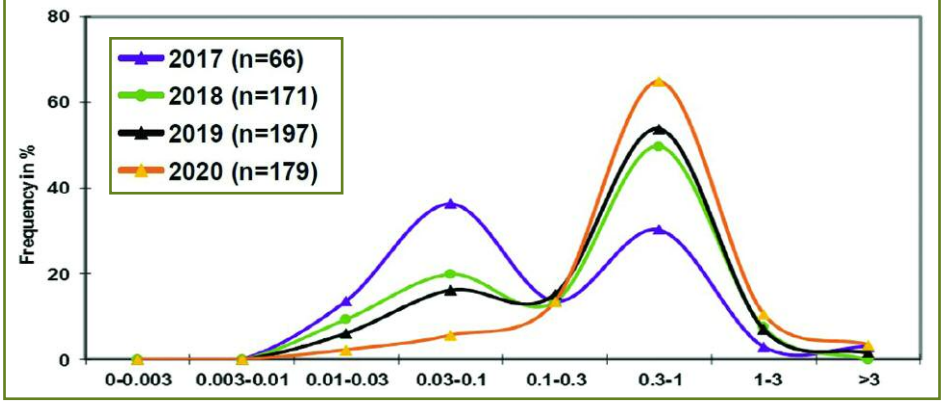
"We have found populations with a ▸

Frequency distribution of ED_{50} of *Zymoseptoria tritici*

2012-2016



2017-2020



Source: BASF internal data; ED_{50} values shown for Xemium for field isolates of septoria collected in UK and Ireland.

▸ relatively high adaptation to Revysol. But while the efficacy of PTZ in the field (in vivo) has continued to decline, Revysol has not. In vitro testing, carried out by both BASF and independently, has indicated a further shift in the sensitivity of isolates to PTZ over the past three years, but by comparison the ED_{50} values for Revysol show it has maintained its superior efficacy."

As with SDHIs, the level of resistance in the UK and Ireland populations is generally higher than it is across the rest of Europe, but the level of resistance specifically to Revysol across Europe is significantly lower than to other azoles, reports Dieter.

One interesting observation has been a difference noted in field isolates tested in France to those gathered in the UK. "Tests indicated higher sensitivity of the French population to PTZ in vitro than when it was tested in the field. But whereas Revysol worked very well in both environments, the PTZ seem to struggle more in France which is surprising. We believe higher levels of UV radiation could be the difference."

Shading tests carried out at ADAS Rosemaund this year have confirmed the difference. Plots were given a single dose of fungicide at flag leaf and half of them were then shaded for 3-4 weeks with septoria levels assessed 55 days after application. The level of infection in the shaded plots treated with PTZ was found to be around half compared with where the crops were left exposed to sunlight. There was very little infection in the Revysol plots, with the shading making little difference.

Another observation has come from profiling ED_{50} values of populations and comparing these across azoles. Work carried out at Aarhus University has noted the sensitivity of Danish and Swedish populations of difenoconazole and tebuconazole correlates well with Revysol. This contrasts significantly with PTZ and epoxiconazole — here the data confirmed what BASF has found, that there's very poor correlation with how Revysol performs.

"But field efficacy work carried out as part of the same study shows very different results from the in vitro tests," says Dieter. "Here Revysol performs significantly higher than all other conventional azoles. There's no doubt there are isolates that are highly adaptive across Europe, but Revysol still works in the field."

While the efficacy of prothioconazole in the field has continued to decline, Revysol has not, notes Dieter Strobel.

So why is this? Dieter points out the target site of demethylation inhibitors (DMI) — the group of fungicides to which the azoles belong — is an enzyme commonly known as CYP51. The most common form of resistance to DMIs is when small mutations occur and accumulate so that the azole binding to the target site is reduced.

"Research has shown that Revysol has a much higher binding affinity to the septoria CYP51 than conventional azoles — on average it is 100 times more powerful. What's more, a very low concentration of Revysol is needed for CYP51 inhibition in vitro."

He refers to this as effective binding, which increases efficacy at one point in time. This is the property Revysol exhibits during in vitro tests, he explains, and is measured in the ED_{50} values. Revysol's ability to bind more tightly, attributed to the unique shape and flexibility of the molecule itself, allows it to overcome a mutation that may thwart conventional azoles, he argues.

But Dieter notes that Revysol also has very low solubility — put it on a leaf and although it's taken up quickly by the plant tissue, there's very little translocation. "So as well as a low concentration required for effective binding, the low solubility means Revysol retains its efficacy over time.

"This is important because septoria takes several weeks to develop in the leaf tissue. During this time, the concentration of other azoles dilutes as they move in the plant xylem." Couple this with the low degradation of Revysol, found in the field studies, and this explains the relatively high in vivo performance, he says.

That's all very well for mutations, but what about other forms of resistance found in the septoria population? These include over-expression, efflux pumps and detoxification — all mechanisms expressed by septoria isolates that are not specific to the azole used.

"Firstly, the presence of very low

concentrations of Revysol due to its poor solubility gives it an advantage as the molecule is less likely to be affected by those pathogen resistance mechanisms," he notes.

"What's more, the proportion of isolates in the European population that exhibit these forms of resistance is currently quite low, and they're believed to carry a fitness penalty. We are monitoring for this and can very quickly identify any substantial changes."

Dieter believes this very robust performance in both in vitro and in vivo tests gives Revysol an extra degree of resilience when it comes to resistance — not only does the new azole currently have substantially better efficacy, it's likely to retain a relatively high level of performance.

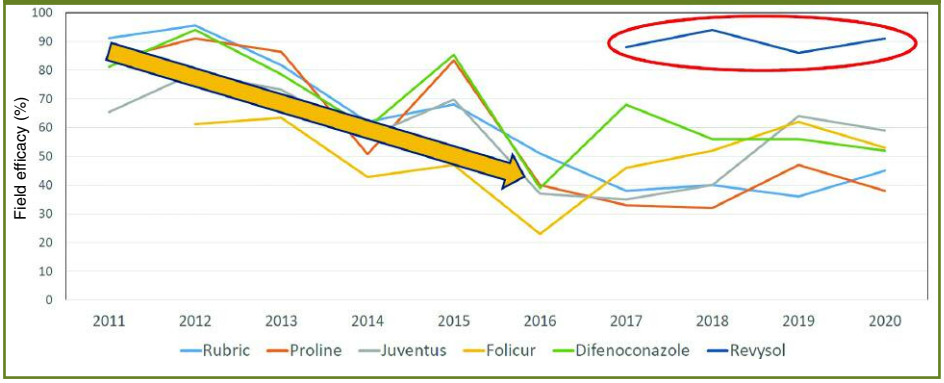
"When we started with Revysol, we set out to find a molecule with a good regulatory profile as well as a high level of efficacy. But the more we work with it, the more surprises it reveals about its performance. However, that's no reason to be relaxed when it comes to stewardship — it's more important than ever to look after chemistry with good efficacy, which means applying it at the appropriate dose, optimum timings and mixing it with other modes of action," he notes.

Rosie echoes this advice. "With the loss of CTL it's important to have effective partners in the tank-mix so they protect each other. Revysol is different from epoxiconazole as it has the properties to deal with a more complex septoria population than in the past, but it still needs protecting as much as the SDHIs."

Using plant genetics is effectively another mode of action, she notes. "There are varieties with good resistance to septoria, but the genetics need protecting, just as much as the chemistry. What's more, you get a good response from effective chemistry with even the most resistant varieties.

"While we'll continue to monitor populations, septoria is a pathogen that will continually seek to out-manoeuvre growers. The strongest line of defence will always be to use the best tools available," concludes Rosie. ■

Field performance of azole fungicides over time



Source: Aarhus University; treatments applied at 2x half dose; Rubric contains epoxiconazole; Proline – prothioconazole; Juventus – metconazole; Folicur – tebuconazole.

Beware the hidden Cougar when selecting for disease

Look across the new AHDB Recommended List of winter wheat varieties and you'll see quite an impressive line-up of disease scores for *Septoria tritici*, especially among the clutch of new Group 3 winter wheats.

But there's some essential information missing from the RL, according to independent plant breeder Bill Angus, that he feels might make you want to question how much you're going to rely on those healthy scores.

"It's their pedigree," he says. "Six out of the eight Group 3 varieties are derivatives of Cougar as is Group 4 (soft) variety RGT Saki. That's a lot of wheats that depend on one set of genetics for their resistance to septoria."

What's more, I suspect that Cougar itself relies on major gene resistance, he points out. "The problem with major genes is that when they capitulate, they go big time. As a grower, you're then left relying on the variety's background resistance. But since the major gene has always masked what the minor genes are contributing, you have no idea whether your variety will then be moderately resistant or completely exposed."

Bill's concerned that in just the same way as growers are losing their choice of chemistry to fight septoria, they're losing the diversity in their genetics. "The problem is that it's not as obvious as an active ingredient losing its approval. You feel encouraged to take your foot off the septoria control, and that's when varietal resistance becomes exposed and the pathogen finds a way round it. Before you know it, lesions are appearing on a variety that should have a septoria score of 7.4."

It's a problem growers are all too familiar with when it comes to yellow rust, he points out. "There are a lot of varieties currently in commercial use that rely on Hereford or Timaru for their apparently high scores. Yet the Hereford yellow rust race is already making inroads into the UK population."

But Bill doesn't dismiss the value of varietal resistance. "Breeders are generally doing a good job of bringing stronger disease resistance into their lines without compromising yield, and that's not easy. You're dealing with a Rubik's cube of traits and every time you try to bring another in, you multiply up the muddle which makes it even harder to line up the ones you want.

"It's up to the grower and agronomist to look behind the headline figures and question the agronomics of a variety on its pedigree before making a choice."

Another strategy he favours for those growing feed varieties is to plant a mixture. "It's a good idea to spread your risk, and growers who make their choices carefully have found they get a number of benefits.

Bill Angus points out there are a lot of wheats on the AHDB Recommended List that depend on one set of genetics for their resistance to septoria.

"Three is a good number, and avoid those with the same or similar pedigrees. Graham, Costello and Theodore might make a good mix for example, although you can run into problems with varieties that don't ripen at the same time or have different heights. So try a small area first," Bill advises.

"But the crucial aspect is not to rely on varietal resistance for disease control — protect the genetics just as you protect the chemistry. It used to be the case that many growers ignored varietal disease resistance and relied too heavily on the chemistry for control. Now we're in danger of going too far in the other direction. The wise grower makes good choices with both their wheat varieties and the chemistry they use to ensure they perform," he concludes.