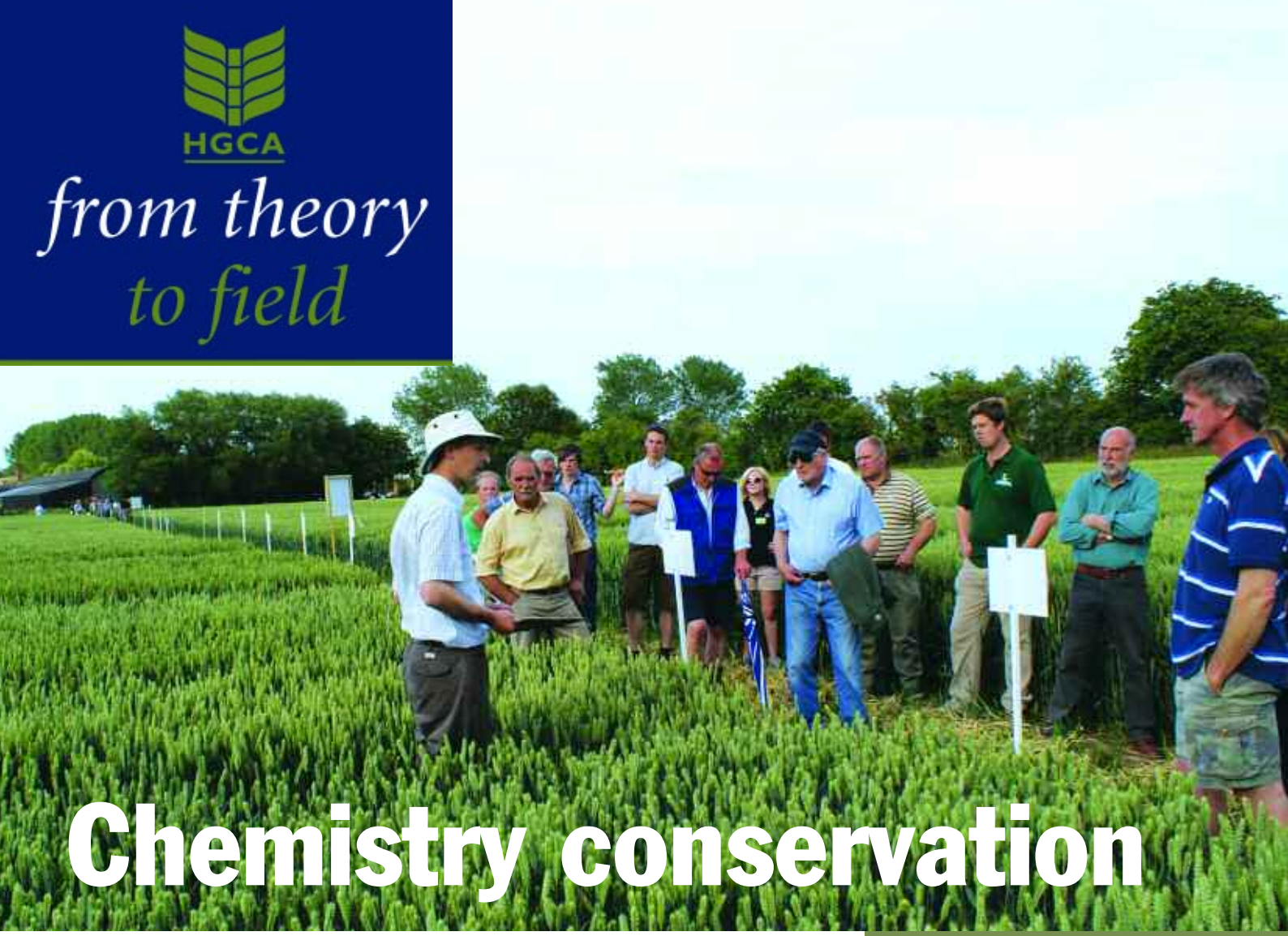
HGCA

from theory to field



Chemistry conservation

A major share of HGCA funding is spent on evaluating new fungicides and assessing the evolving picture on fungal sensitivity to existing products. *CPM* finds out how the work is helping to shape spray programmes.

By Tom Allen-Stevens

Of all the work HGCA undertakes, its fungicide evaluation research is the most valued by levy payers, according to feedback, second only to the Recommended Lists. But in recent years, the rise of azole resistance and introduction of SDHIs have thrown even greater weighting on its findings.

"The new fungicide performance work is actually a rolling project that has been carried out since the early 1990s. It's the one growers look out for most, and is a major hook for the HGCA Agronomist Conference in Dec," notes Dr Paul Gosling of HGCA. "It provides objective, independent data on the performance of individual products side-by-side — the *Which?* guide to fungicide performance in the UK."

Distributors and chemical manufacturers do similar evaluation trials, he recognises. "But they don't always compare the leading products side by side, and results sometimes come with a marketing message. The HGCA trials provide basic information on all the leading products that growers and agronomists can interpret to build their spray programmes."

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Arms length

HGCA works alongside chemical manufacturers, who provide new unregistered fungicides for testing and any technical support needed. "They're kept at arms length, however. The project is overseen by the Fungicide Working Group, that contains no agchem representatives. Manufacturers see the results prior to publication, but can't veto anything as long as it's technically correct."

Related to this research is work looking at azole-sensitivity shifts. "Azole chemistry remains the backbone of most growers' disease control programmes, but it's well established that septoria is now less sensitive to azoles. The azole work is about understanding the molecular mechanisms responsible for this shift, and how the population of septoria isolates is changing."

Similarly, HGCA is a partner in a LINK, industry-funded project focused on SDHIs. This is investigating in the lab how resistance to SDHIs could develop in the field, with the aim of building spray programmes that'll preserve the efficacy of this new chemistry.

"When strobilurin chemistry fell over, there was no monitoring and little awareness among growers. If we can

understand the resistance mechanism more clearly, we can manage the chemistry with a longer-term gain in mind, rather than just the short-term benefit," notes Paul Gosling.

Comparing the benefits of the SDHIs against the older chemistry is an essential part of the fungicide performance work, says Jonathan Blake of ADAS, who leads the project.

Underlying efficacy

"We need to have that data before a new product comes to market so growers can have the confidence to switch. The trials are designed to pull apart the products and identify their underlying efficacy so we can identify their strengths and weaknesses."

Crops are treated at various rates of product, from a quarter dose to double dose (although such high rates aren't allowed in commercial practice), on their own and in mixtures. There are trial sites across the UK in England and in Scotland, while results of similar tests, run by Teagasc in Ireland, are also included.

The SDHIs have featured in trials since 2008. But what researchers have noticed since then, about their performance where septoria is the main target, could have worrying implications for growers, notes Jonathan Blake.

"When we started with SDHIs, azoles were doing two thirds of the disease control, with SDHIs contributing just the extra third. But there's been a shift in balance over the last couple of years and it's now the other way round. So SDHIs

Jonathan Blake has seen a shift in balance over the last couple of years, with SDHIs now relied on to provide two thirds of disease control.

The new fungicide performance project provides growers with the Which? *guide to fungicide products, says Paul Gosling.*

are now an essential part of maintaining control of septoria."

This puts greater dependency on the chemistry, believes Jonathan Blake, which will leave a big hole in spray programmes if septoria develops resistance to SDHIs.

At the heart of the problem is the gradual slide in efficacy of the azoles, he says, and this has been tracked through the trials over the years. It was the long-term nature of the work that allowed researchers to pick back over old data and identify trends.

"In 1995, a quarter rate of epoxiconazole ▶

Trials are treated once at various rates of product, from a quarter dose to double dose, on their own and in mixtures.

▸ achieved 75% control in a protectant situation but by 2012, that had more than halved to just 35%."

2012 proved an instructive year for pulling apart the SDHIs, continues Jonathan Blake. "Until 2011, Aviator Xpro (bixafen+ prothioconazole) and Adexar (fluxapyroxad+ epoxiconazole) performed the same on a l/ha basis, even though this was four fifths of the label rate of Aviator and a half label rate of Adexar. But in 2012, you needed more than 1 l/ha of Adexar to get the same performance on septoria as 1 l/ha of Aviator."

For the past two years, a new element has been added to the trials, with the spray-timing flexibility of various products now under scrutiny. "We're applying products before and after their ideal timings to see how they differ in flexibility. We'll have the first results in 2013."

Meanwhile, more detailed work to improve stewardship programmes for SDHIs has been carried out through a major, joint industry, LINK-funded project. Part of this work has been to test over a thousand isolates per year of *Mycosphaerella graminicola, the* pathogen that causes septoria.

Risk of resistance

"The good news is that so far we haven't detected any resistance to SDHIs in samples we've tested from the field," reports Dr Bart Fraaije of Rothamsted Research who's leading the project. "But the risk of resistance is very real."

SDHIs block the function of succinate dehydrogenase, an important enzyme required for mitochondrial respiration, which leads to the death of the fungus. The fungicide is designed to bind to specific sites in the Sdh complex.

But the docking of these fungicides is very precise, so just a slight shift in the structure of the complex, and the SDHI may no longer bind to its target site, meaning the pathogen will then be resistant.

So why do the scientists believe the risk of resistance is so high? "We've altered some isolates in the lab through exposure to UV radiation. We've tested these mutants and some of them are highly resistant to SDHIs. So we know the potential for resistance is there and there's a serious risk similar mutations will occur in the field."

It's possible that resistant isolates do exist in the field, but have a fitness penalty and die out quickly or fail to multiply, he

So far no resistance to SDHIs has been found in samples tested from the field but the risk of resistance is very real, says Bart Fraaije.

Robust spray strategy based on good information

For Andrew Gilchrist of Scottish Agronomy, the HGCA fungicide evaluation work is a valuable reference point for the data generated from the company's own trials. "The fungicide performance curves are useful, and the results generally confirm what we're finding in our own trials. It gives you extra confidence in the products you're planning to use.

"But the trials aren't a practical comparison — they show the individual performance at one hit for different rates, which isn't a real-world comparison of how they'd behave in a programme."

And the gradual decline in azole sensitivity is an issue that he's had to build into spray programmes. "We're applying higher and higher dose rates in order to maintain the same level of efficacy. It's useful to have the data coming though to inform us how that shift is evolving, so that we can take it on board in practical terms."

The data also has its limitations, however. "In Scotland, as we know we're dealing with slightly different populations to the rest of the UK, regional efficacy trials, such as our own, are important to provide a complete picture of the local situation."

Andrew Gilchrist relies on good external information he can trust to add to in-house trials results as the basis for building spray programmes."

In practical terms, Andrew Gilchrist has adapted spray strategies through adopting SDHIs early on and using them to best effect within the programme. Multi-site active chlorothalonil (CTL) also plays a crucial role, while triazole rates are always kept at a robust robust.

"What worries me is that some straight SDHI material has been registered. The temptation is there to use these without appropriate mixing partners or to try to get away with the bare minimum of dose rates, with no regard for resistance developing."

Meanwhile his strategy on wheat will be a "solid" T0, that sees a triazole with other partners protect against early rust and septoria. "CTL will play a key role, particularly in co-formulations."

The T1 will be boscalid or prothioconazole-based with more CTL. "At T2, we'll use one of the new-generation SDHIs. Aviator arguably showed a slight edge over Adexar and Seguris (isopyrazam+ epoxiconazole) in 2012, both in the HGCA trials and ours, but it could be the other way round this year. What we choose will depend partly on what's in the field, partly on the product's back-catalogue history and partly on price — all three do a pretty good job."

The T3 will be another solid application, probably based on prothioconazole, says Andrew Gilchrist. "Most of what we recommend is based on our extensive trials, but it's essential to have good external information you can trust to build into the mix."

says. But further testing in the lab has revealed the mutant strains do produce spores. "So there are no measurable fitness costs associated with these mutations," points out Bart Fraaije.

The work with the mutations, along with docking studies to establish how SDHIs bind to their target site, will help researchers develop a diagnostic test. "We know quite a lot about the mutations that will cause resistance, so should be able to develop a test that'll pick it up quickly if it's found in the field.

"The work we've done may also help manufacturers develop the chemistry — it's not a cut-off switch in quite the same way as resistance to strobilurins. The SDHI fluopyram can control some mutants that other molecules can't, for example. So if resistance develops, the chemistry won't necessarily be ineffective."

The relationship between azoles and SDHI sensitivity is also being examined. "We hope to find certain situations in which we can slow down the rate of azole selection using SDHIs." Trials at ADAS and SRUC are looking at specific fungicide mixes and rates, and how these influence particular isolate populations. "The work done so far suggests that if we can get the chemistry and the loading right, we can take pressure off the azoles."

Another HGCA-funded project is monitoring the mechanisms of azole resistance. Azoles work by inhibiting an enzyme used by the pathogen to form cell membranes. But selection pressure by increasingly effective azoles has led to mutations in the target protein, known as CYP51.

Up to eight mutations have been found in the CYP51 protein, conferring higher levels of insensitivity towards epoxiconazole and prothioconazole.

"This is the most common mechanism of azole resistance," explains Bart Fraaije. "The first mutations were identified in samples from the late 1980s. An amino acid at a particular site in the protein is switched, which has the effect of decreasing sensitivity to an azole or group of azoles.

"Initially there was just a single mutation, but we're now finding up to eight changes together in a single isolate. This is conferring higher levels of insensitivity towards the main azoles used — epoxiconazole and prothioconazole."

Overexpressing strain

But then in 2009, scientists identified a new resistance mechanism. They found *M. graminicola* could produce more, or overexpress, the CYP51 protein, making it less sensitive to all azoles. "The CYP51 overexpressing strain was found more often in 2011 and 2012," reports Bart Fraaije.

What's more, a third mechanism has been reported, known as enhanced efflux. This is where the fungus pumps azole out of its cells. "The mechanism has been suggested to confer a multiple drug resistance (MDR) phenotype, across azoles and to non-azole fungicides. But MDR strains have not been found in the UK."

With super mutations in the field, then, what hope does modern chemistry have against it? "Some of the most extreme phenotypes aren't very common, so there could be a fitness penalty. And sometimes the mutations causing resistance to some azoles, increase sensitivity to others — tebuconazole and prochloraz sensitivity in the most recent variants were less affected, for example."

Some M. graminicola *isolates, altered in the lab through exposure to UV radiation, have been found to be highly resistant to SDHIs.*

Current work in this area is focused on fungicide sensitivity testing of the extreme CYP51 variants and overexpressing strains on wheat plants.

"But the best practice remains to keep rates high and to use different azoles across the programme, along with fungicides with different modes of action, such as multi-site inhibitors. The decline is gradual, and azoles still form a key part of the armoury. But the fungus is evolving and coping better, so it's up to growers year on year to adopt a more robust approach to septoria control." ■

Research round-up

HGCA project 3734, New fungicide performance on winter wheat, runs from Sept 2010 to March 2014. Its aim is to provide an independent assessment of the relative performance of new and old fungicides against the economically important foliar diseases of winter wheat. Its total cost is £450,896 funded by HGCA. The research is carried out by ADAS, NIAB TAG and SRUC.

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, runs from April 2011 to March 2014. Its aim is 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 total cost is £60,000 funded by HGCA. The research is carried out by Rothamsted Research.

HGCA project 3517, Improved tools to rationalise and support stewardship programmes for SDHI fungicides to control cereal diseases in the UK, runs from Jan 2010 to Dec 2013. Its aim is to provide a scientific framework for sustainable use of the newly developed succinate dehydrogenase inhibitors (SDHI) and their mixing partners to maintain or enhance cereal production in the UK. Its total cost is £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.