PROJECT REPORT No. 275

MAINTAINING THE EFFECTIVENESS OF DMI FUNGICIDES IN CEREAL DISEASE CONTROL STRATEGIES

MARCH 2002

PRICE: £4.60

PROJECT REPORT No. 275

MAINTAINING THE EFFECTIVENESS OF DMI FUNGICIDES IN CEREAL DISEASE CONTROL STRATEGIES

by

D HOLLOMON[1], L COOKE[2] and T LOCKE[3]

[1]IACR – Long Ashton, Bristol BS41 9AF

[2]The Queen's University of Belfast, Department of Applied Plant Science Agriculture & Food Science Centre, Newforge Lane, Belfast, County Antrim BT9 5PX

[3]ADAS Rosemaund, Preston Wynne, Herefordshire HR1 3PG

This is the final report of a three-year project which started in April 1998. The work was funded by a grant of £202,839 from HGCA (project no. 1406).

CONTENTS

ABSTRACT

A series of field trials carried out over three years 1997 to 2000 in different parts of the UK, formed the basis of an evaluation of fungicide mixtures as anti-resistance strategies to maintain effectiveness of DMI (triazole) fungicides against Septoria disease, caused by *Mycosphaerella graminicola (=Septoria tritici)*. Trials focussed on mixtures of either epoxiconazole (Opus) or tebuconazole (Folicur) with azoxystrobin (Amistar), at doses ranging from recommended rate to ¼ field rate. Despite the wide range in sensitivity to DMIs in *M. graminicola,* monitoring sensitivity of isolates from these trial sites to several DMIs showed no changes in sensitivity during the three years, regardless of the rate of fungicide applied. Epoxiconazole was the best fungicide when used alone, but adding a strobilurin improved disease control, and yields, especially when mixed with less effective DMIs than epoxiconazole. Reduced rates of both mixture partners were sufficient to achieve these benefits.

In a separate study, contour maps linking samples with similar sensitivities to cyproconazole, flusilazole and flutriafol highlighted the lack of uniformity of DMI sensitivities within a *M. graminicola* population. Despite an underlying similarity between these three DMIs, there were significant differences in sensitivity levels between them, and this probably reflected several different mechanisms contributing to sensitivity levels. Analysing these data using the geo-statistical technique, *"kriging"*, indicated that samples should be collected at least 20 metres apart to ensure they were independent of each other. This provided a guide to sampling protocols needed to define DMI sensitivity distributions, but after selection with epoxiconazole too few lesions were available to construct maps with the same precision as generated prior to spraying. Although there was no evidence of any change in the contour maps following selection, this illustrates a feasible approach to monitoring the effects of anti-resistance strategies on a field scale, without replicating the treatments.

Applying PCR diagnostic techniques to follow the development of *M. graminicola* revealed that azoxystrobin had significant curative as well as preventative effects, and these lasted for up to one month after treatment. By slowing down development of the pathogen, azoxystrobin enhanced the curative activity of the DMI partner. This not only ensures that DMI/strobilurin mixtures are a good anti-resistance strategy, but the prolonged action of the mixture means that spray intervals can be increased, such that a two-spray programme should be sufficient to adequately control Septoria.

SUMMARY

Fungicide resistance increases growers' costs not only through lost production, but also through the need to use more expensive replacement fungicides. For instance, resistance to MBC fungicides in cereal eyespot in the early 1980's generated an annual cost to growers of £25m, and some of elements of the increased costs continues today. Loss of DMI fungicides (also called azoles) which block sterol biosynthesis as 14α **D**e**M**ethylase **I**nhibitors), because of resistance in *Mycosphaerella graminicola* (Septoria leaf blotch) would be no less costly. All major foliar and stem base diseases of cereals have been subjected to selection with DMIs for more than 20 years, and resistance has developed in some of these but not, as yet, in Septoria. Consequently, there is a need to explore anti-resistance strategies which will prolong the durability of DMI fungicides.

The aims of this project were to:

- Identify suitable mixture partners to manage DMI resistance in *Mycosphaerella graminicola* in wheat.
- To provide information on anti-resistance management for the DESSAC programme, with particular emphasis on DMI/QOI (=strobilurin) mixtures.
- To explore novel statistical methods to chart fungicide sensitivity changes in field trials without need for replication.

Micro-titre plate based assays allowed the monitoring of sensitivity, to cyproconazole, epoxiconazole, flutriafol, flusilazole, and tebuconazole, in thousands of isolates from wheat crops receiving a range of these fungicide treatments. Standard "sensitive" and "resistant"isolates were included in each assay, and growth was assessed after 7 days in a micro-titre plate reader at 540nm. Analysis of survey results from England, Wales and Northern Ireland showed no shift in sensitivity to these azoles during the three growing seasons, 1998 to 2000. Performance was generally good, and isolates were often difficult to find in some treated plots. Indeed, even where lesions were collected, recovering live fungal material from them was often difficult, especially after spraying with epoxiconazole (Opus) or azoxystrobin (Amistar).

Extensive monitoring of isolates collected from 40 different sites within a 5ha crop of winter wheat (cv. Riband) produced contour maps for each fungicide linking areas where sensitivity values were the same. This approach revealed considerable diversity in sensitivities within the *M. graminicola* population, and exposed different cross-sensitivity patterns between flutriafol, cyproconazole and flusilazole. Applying the geo-statistical technique "*Kriging*" to these data defined a protocol which ensured that samples were collected sufficiently far apart to be independent of each other. The technique also allowed comparison of these sensitivity contour plots before and after fungicide treatments, without need for replication. Spraying the whole crop with Opus did not significantly change the contour plots.

Using a quantitative PCR diagnostic technique developed in a previous HGCA funded project (HGCA Report No. 245), allowed us to follow the development of *M. graminicola* in field crops prior to symptoms, and assess the effects of different fungicide treatments on the spread of the pathogen within the wheat plant. Under field conditions, none of the fungicides delayed the initial detection of the pathogen in the

upper three leaves of the wheat canopy, but they did slow subsequent development. This work revealed that azoxystrobin had a significant curative effect, and this effect lasted for up to one month after spraying. Slowing the pathogen down in this way allowed the curative action of DMIs to persist longer, and especially for those DMIs less effective than epoxiconazole.

These observations were reflected in the performance of DMI/azoxystrobin mixtures in field trials, where mixtures of azoxystrobin with tebuconazole performed as well as mixtures with epoxiconazole. Half rate azoxystrobin was generally sufficient to achieve these benefits. Combining these data with information on rainfall patterns, cultivar susceptibility to *M. graminicola*, and various agronomic factors within the DESSAC programme should improve decision making in the management of Septoria disease.

The outcome of this project produced the following conclusions:

- Evaluating the effectiveness of anti-resistance strategies can be achieved in the whole crop situation without replication, provided samples are collected sufficiently far apart to be independent of each other.

- Monitoring *M. graminicola* isolates from crops in Northern Ireland, England and Wales has provided no evidence of a decline in sensitivity to DMI fungicides in the three years 1998 – 2000.

- Combining the curative activities of azoxystrobin and DMIs provides a good anti-resistance strategy. The essentially fungistatic action of azoxystrobin ensures that individuals with reduced sensitivity, and which grow slowly, are exposed longer to the strong curative action of DMIs such as epoxiconazole.

- The prolonged action of the mixture means that spray intervals can be increased, and a two-spray programme should be sufficient to adequately control Septoria leaf blotch.

INTRODUCTION

Following their introduction in the early 1980's, broad-spectrum DMI fungicides quickly became key tools in controlling cereal diseases. Commonly referred to as "azole fungicides", DMIs block fungal sterol biosynthesis as 14-α-**D**e**M**ethylation **I**nhibitors. At least 13 different DMIs are currently registered in the UK for use on cereals, and in wheat an average of 2.5 sprays of DMIs each season are applied between GS 25 and GS 71(Hardwick *et al.,* 2000). Many DMIs have good systemicity and are used as seed treatments to control foliar diseases, and more recently the root pathogen *Gaumannomyces graminis*, which causes "take-all" disease. DMIs are seldom used as single products, but generally as mixtures with products with different modes of action, such as MBCs, morpholines, anilinopyrimidines, and especially strobilurins (QOIs). Application rates in mixtures are usually around half the rate recommended for use of DMIs alone.

All the major cereal diseases have been subjected to selection with DMIs for over 20 years and, as a result, resistance has developed in some pathogens to the extent that their performance against wheat and barley powdery mildews, and *Rhynchosporium*, has been reduced. But DMIs continue to perform well against *Mycosphaerella graminicola* (= *Septoria tritici*), and despite the wide range insensitivities to DMIs in natural populations of this important wheat pathogen (Herman & Gisi, 1994), selection has had little impact in increasing the frequency of resistant strains (Metcalfe *et al.*, 1998). Performance against cereal rusts has also remained good, although some decline in sensitivity has been detected in both yellow and brown rust of wheat (Bayles, personal communication, 2001). DMIs such as prochloraz are used to control the cereal stem-base eyespot pathogen (*Pseudocercosporella* sp.), and although resistance has caused control failures in France (Leroux *et al*., 2000), this has not been the case in the UK.

A cornerstone of anti-resistance strategies is the use of fungicide mixtures. Not only should mixture partners have different modes of action, but ideally should act together throughout development of the target pathogen. Consequently, good understanding of the biological modes of action of mixture partners is essential to provide the framework needed to define which partners to use, and at what dose rates. This is especially critical for control of *M. graminicola* with DMIs, since each DMI varies significantly in its curative properties against this disease. Strobilurin fungicides, which are considered to be largely preventative, provide a new dimension to mixtures, and defining the extent of their curative activity will guide which DMI mixture partners are best.

Infection and development of *M. graminicola* have been particularly difficult to follow microscopically during the early biotrophic and latent phase of this pathogen. But recent developments in the detection and quantification of *M. gramincola* have provided both immunological (Mittermeier *et al*., 1990; Joerger, *et al., 1992*) and PCR (Fraaije *et al.* 1999) diagnostic tools to follow development, and the effects different fungicides have on early infection. PCR techniques are particularly powerful since they allow detection of the pathogen within three days of infection starting. These techniques can follow developments in field crops, and are not restricted to growth room and glasshouse studies. Clearly, development of anti-resistance spray

programmes aimed at preserving the activity of DMIs against *S. tritici* would benefit from defining the curative and preventative activity of potential mixture partners.

Evaluating the usefulness of different anti-resistance strategies requires accurate monitoring of any changes in the distribution of fungicide sensitivity within a pathogen population in response to selection. This requires understanding how sensitivity differences are distributed within a pathogen population, since this influences the sampling protocol needed to achieve a true picture of sensitivity changes. In most studies reported so far, the effectiveness of anti-resistance strategies has been judged from shifts in the mean sensitivity of several isolates collected within each treatment plot. Values from replicate plots allow statistical analysis of these results.

To develop an alternative approach which does not require replication, and which would be well suited to monitoring fungicide sensitivity in commercial crops rather than replicated field experiments, we plan to apply the geo-statistical " Kriging" technique, which is widely used by the mining industry to assess the metal content of ore-bodies, from non-random borings. Kriging has also been used to map soil fertility (Webster & McBratney, 1987). The technique can be used to define the distance needed between sample points to ensure that independent samples are collected. Despite the apparent relevance of this statistical technique to many plant disease epidemiological questions, few attempts have been made to explore the use of Kriging in this context.

OBJECTIVES

1. To identify suitable mixture partners to manage DMI resistance in *Septoria tritici (Mycosphearella graminicola)* in wheat.

2. To provide information on anti-resistance management for the DESSAC programme, with particular emphasis on DMI/strobilurin mixtures.

2. To explore novel statistical methods to chart fungicide sensitivity changes in field trials without need for replication.

MATERIALS AND METHODS

In vitro testing of *Mycosphearella graminicola*

Lesions were cut from field samples so that some green leaf tissue (3 cm) remained around each lesion. Each leaf piece was then stuck into distilled water agar in Petri-dishes (9cm diam.), such that the lesion was not in contact with the agar. The uncovered dishes were placed in trays lined with moist filter paper, and a lid placed over the tray. Trays were incubated on a laboratory bench for 48 hours until pycnidia began to ooze. Individual cirrhi were picked off with a sterile needle and transferred to 10ml sterile glucose-yeast liquid medium (Yeast Extract, OXOID No. L21 0.2% w/v; Glucose 1.0% w/v; Chloramphenicol 100 ppm). Pycnidiospore suspensions were left overnight at 4^{o}C to reduce the amount of bacterial contamination.

Stock solutions of each fungicide were prepared in ethanol and 10µl was pipetted into wells of sterile micro-titre plates, to generate for each isolate tested a dilution series of 3, 1, 0.33, 0.11, 0.037, 0.011 µg, together with a control (none). A high dose (30µg) was used to "blank" in the micro-titre plate reader, since no fungicide totally prevented germination. Ethanol was allowed to evaporate, before 200µl of the spore suspension was pipetted into each well. Duplicate wells were prepared for each concentration and each isolate tested. Lids were placed on each plate and sealed with parafilm. Plates were incubated for 7days at 17^{o} C with constant rocking, and growth measured in a plate reader at 540nm. Visual assessment identified wells where growth was not solely caused by *M. graminicola*.. Fungicide sensitivity was determined either as **M**inimum **I**nhibitory **C**oncentration (MIC), or as ED_{50} values calculated from a dose response relationship. A standard sensitive (RL2) and "resistant" (S27) strain were included in each micro-titre plate.

Contour mapping of *M. graminicola* sensitivity levels within a wheat crop

Leaf samples were collected from 40 different sites within a crop of *Riband* winter wheat before and after applying epoxiconazole (Opus) at GS 42. Sensitivity assays were carried out in micro-titre plates as described in the previous section, and at least five isolates were tested from each sample. Assays included three DMI fungicides – flutriafol, flusilazole and cyproconazole. Contour maps were drawn by linking sample sites with the same average MIC values. Appropriate statistical techniques were used to define any significant differences between contour maps, and a Kriging approach to identify the distance between independent samples

Quantitative PCR protocol

For each field sample, ten leaves were ground and the DNA extracted according to the method described in Fraaije *et al*.(1999). 100ng of total DNA was used as template in the subsequent PCR , and known amounts of *M. graminicola* DNA were added to healthy wheat leaf DNA as standards, which were then run simultaneously with the samples. Primers E1 and S1SP2R (Fraaije *et al* 1999) were used to amplify specifically a 496bp fragment from the β-tubulin gene of *M. graminicola*, as a control of for the PCR reaction. After 35 cycles *M. graminicola* DNA was quantified by measuring the fluorescence produced by the cyanine dye PicoGreen, using a calibration curve generated from standards. Healthy leaves were extracted after every 20 samples, and used as negative controls to ensure the absence of cross contamination between samples. Amounts of *M. graminicola* DNA are expressed in pg of fungal DNA per 100 ng of wheat DNA.

Field trials

Examination of preventative and curative effects.

After a preliminary experiment in 1998 using a two-block randomised design, in the following two years more detailed experiments involving four replicates were carried out . Plots (4m^2) were sown in mid-October with the cultivar "Brigadier", which is highly susceptible to *M. graminicola*. Ten leaves from each leaf layer were sampled fortnightly from GS 37 through to GS 77 and the mean necrotic area per sample

assessed visually. The following fungicides were applied at GG 32 in 1998 when leaf 2 was fully emerged, and at GS 37 in 1999 and 2000 when the flag leaf was completely emerged:
Azoxystrobin (250g a.i. l^{-1}) as "Amistar".
Epoxiconazole (125g a.i. l^{-1} as "Opus"
Chlorothalonil (500g a.i. l^{-1} as "Bravo 500"
Fungicides were applied in a spray volume equivalent to 300 l ha^{-1}.

Evaluation of anti-resistance strategies

A core component of this project was the evaluation of different fungicide mixtures as possible anti-resistance strategies. Consequently, in each year field trials were carried out in Northern Ireland (DANI), the West Midlands (ADAS) and at Long Ashton. The various treatments are too numerous to mention individually, but mixtures of a DMI (tebuconazole or epoxiconazole) and strobilurin (azoxystrobin; in one trial at Long Ashton an experimental formulation of Famoxate), were used with a number of different dose rates of each mixture partner. Table 1 shows the treatment profile of one trial. Cultivars commonly used in these trials included *Riband* and *Consort*. The two sprays were applied at GS 31-33 (T1) and at GS 41-42 (T2), although in 1999 adverse weather conditions delayed treatment of some trials until GS 59.

Table 1. fungicide treatments at two timings (product and dose ha^{-1})

Treatment	Spray1	l/ha	Spray 2	l/ha
1	None		None	
2	Folicur	1.0	Folicur	1.0
3	Folicur	0.75	Folicur	0.75
4	Folicur	0.5	Folicur	0.5
5	Folicur	0.25	Folicur	0.25
6	Opus	1.0	Opus	1.0
7	Opus	0.75	Opus	0.75
8	Opus	0.5	Opus	0.5
9	Opus	0.25	Opus	0.25
10	Amistar	1.0	Amistar	1.0
11	Amistar	0.5	Amistar	0.5
12	Amistar + Folicur	0.5 + 1.0	Amistar + Folicur	0.5 + 1.0
13	Amistar + Folicur	0.5 + 0.75	Amistar + Folicur	0.5 + 0.75
14	Amistar + Folicur	0.5 + 0.5	Amistar + Folicur	0.5 + 0.5
15	Amistar + Folicur	0.5 + 0.25	Amistar + Folicur	0.5 + 0.25
16	Amistar + Opus	0.5 +1.0	Amistar + Opus	0.5 +1.0
17	Amistar + Opus	0.5 + 0.75	Amistar + Opus	0.5 + 0.75
18	Amistar + Opus	0.5 + 0.5	Amistar + Opus	0.5 + 0.5
19	Amistar + Opus	0.5 + 0.25	Amistar + Opus	0.5 + 0.25

RESULTS

Fungicide sensitivity monitoring

One feature that soon became apparent during the first seasons' survey was that many lesions failed to produce viable pycnidiospores. This was explored by detailed analysis of the number of pycnidia in lesions, and the number of pycnidia producing viable pycnidiospores. Azoxystrobin, and especially epoxiconazole, mainly reduced the number of lesions with pycnidia (Table 2), although both fungicides also affected the viability of pycnidiospores. Flusilazole had little effect on pycnidiospore production. Monitoring the sensitivity to flusilazole and cyproconazole for samples from over 3,000 lesions collected from field trials between 1997 and 1999 showed no significant shift in mean sensitivity (Figure 1), although the sensitivity range was somewhat broader in 1999 than earlier . Only cyproconazole was assayed in 2000, but again there was no change from the previous year in mean sensitivity, or the range of sensitivities (Figure 2).

A similar situation was observed for isolates collected and assayed in Northern Ireland. Sensitivity to epoxiconazole showed no clear change between 1998 and 2000 (Figure 3). Although few isolates were obtained from treated crops, especially in 1998, there was no evidence that treatment with either tebuconazole or epoxiconazole, decreased the sensitivity to these DMI fungicides (Figure 4). Various mixtures with azoxystrobin (Amistar) had little overall effect on DMI sensitivities.

Contour mapping

Prior to any fungicide treatment the distribution of DMI sensitivities was not uniform across the field (Figure 5). Furthermore, although there was an underlying low level of cross sensitivity between the 3 DMI fungicides, especially in the part of the crop where the pathogen was most sensitive, contour maps for each fungicide were significantly different ($p = 0.05\%$). In one area samples showed reduced sensitivity to cyproconazole and flutriafol, but less so to flusilazole. Elsewhere within the crop, reduced sensitivity to flusilazole occurred, but was not necessarily linked to reduced sensitivity to the other two DMIs.

The number of isolates available for testing after treatment with epoxiconazole was greatly reduced and, consequently, contour maps were less precise. Nevertheless, there was no evidence of changes in these contour maps as a result of selection (data not shown).

Table 2

Development of Septoria lesions and pycnidia on untreated and fungicide treated wheat cv Riband

Treatment	Number of lesions	% of lesions containing pycnidia	% of lesions containing spores
Untreated	88	54.65	47.86
Opus (0.25)	180	5.55	2.78
Sanction (0.25)	88	51.14	34.09
Expt. Strob. (0.5)	113	20.35	13.27

Figure 1

Sensitivity of *Mycosphaerella graminicola* to DMI fungicides (1997 - 99)

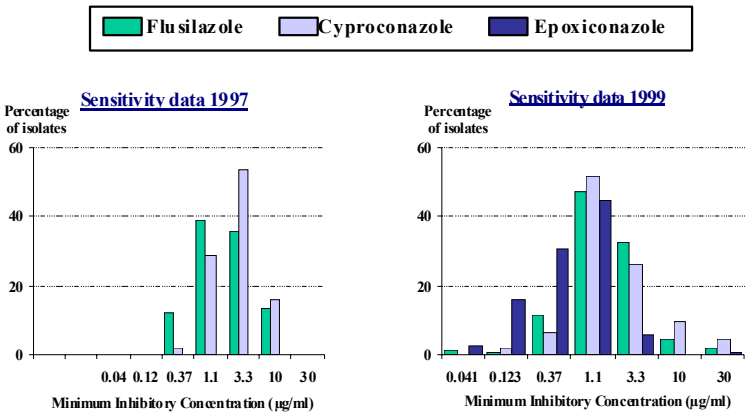
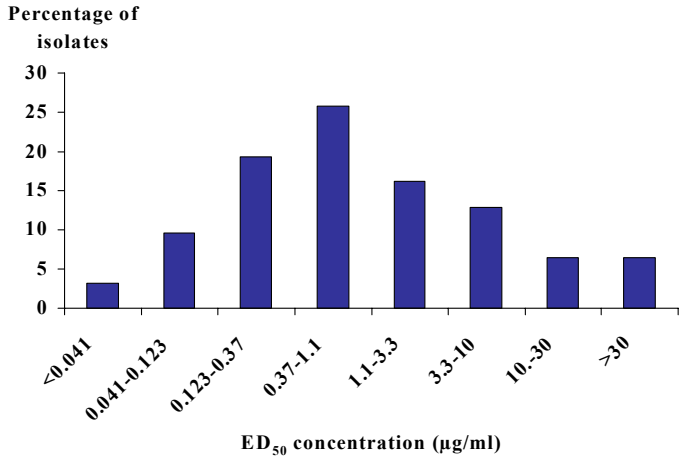


Figure 2

Sensitivity distribution of *Mycosphaerella graminicola* to cyproconazole in 2000



The contour maps identified several foci where sensitivity, especially to cyproconazole, was reduced, which suggests that isolates within each focus are not independent of each other, but are derived from a single less sensitive isolate either generated *in situ* by mutation, or arriving from elsewhere within the crop, or from totally outside it. In this cropping situation, a Kriging analysis of these data indicates that samples must be collected at least 20 metres apart to be independent of each other (Parker, personal communication, 2001), and that each focus could result from a separate infection event.

Effect of fungicides on the spread of *M. graminicola* in different leaf layers

PCR showed that the fungicides were applied as curative treatments on leaf 3, and preventatively on leaves 2 and 1 (Flag leaf; Figure 6). *M. graminicola* DNA was detected three weeks before symptoms, and overall there was a good correlation between PCR quantification and subsequent appearance of symptoms. None of the fungicides delayed the first PCR detection of the pathogen in any leaf layer, but azoxystrobin decreased the amount of fungal DNA during the early stages of infection, whereas epoxiconazole especialy reduced DNA levels after initial infections had been established. Chlorothalonil was only preventative and its effects largely restricted to leaf 2 , which was healthy at the time of treatment. Yield benefits were obtained with all three treatments, and in 1999 a single treatment at GS 39 with azoxystrobin or epoxiconazole doubled yield.

Field evaluation of DMI/strobilurin mixtures

In all field trials carried out during this project Septoria was the main disease present, especially by GS 65, when levels on leaf 2 were seldom less than 20% in untreated plots. All fungicides reduced disease levels, and epoxiconazole generally gave better

disease control than tebuconazole (Figure 7). Increasing the fungicide rate improved disease control, especially where DMIs were used alone. Adding azoxystrobin improved Septoria control still further, although not always significantly when epoxiconazole was the mixture partner. Famoxate also improved Septoria control when added to the DMI flusilazole (data not shown). In addition, strobilurins increased the green leaf area and longevity of the upper leaves, although half rate azoxystrobin was sufficient to achieve this effect.

With the exception of the lowest (¼) rate all DMIs increased yields (Figure 8), and these yields were further increased by the addition of azoxystrobin. On some occasions at the English sites the two-spray mixture programme actually doubled, yields, although more commonly yield increases were in the order of 2 tonnes ha^{-1} The yield benefits from azoxystrobin as a DMI mixture partner were most pronounced when mixed with the less effective DMI, tebuconazole (Figure 9). Half-rate azoystrobin was generally sufficient to achieve these benefits.

Figure 3 Sensitivity to epoxiconazole of *Mycosphaerella graminicola* From Northern Ireland field trial sites, 1998-2000

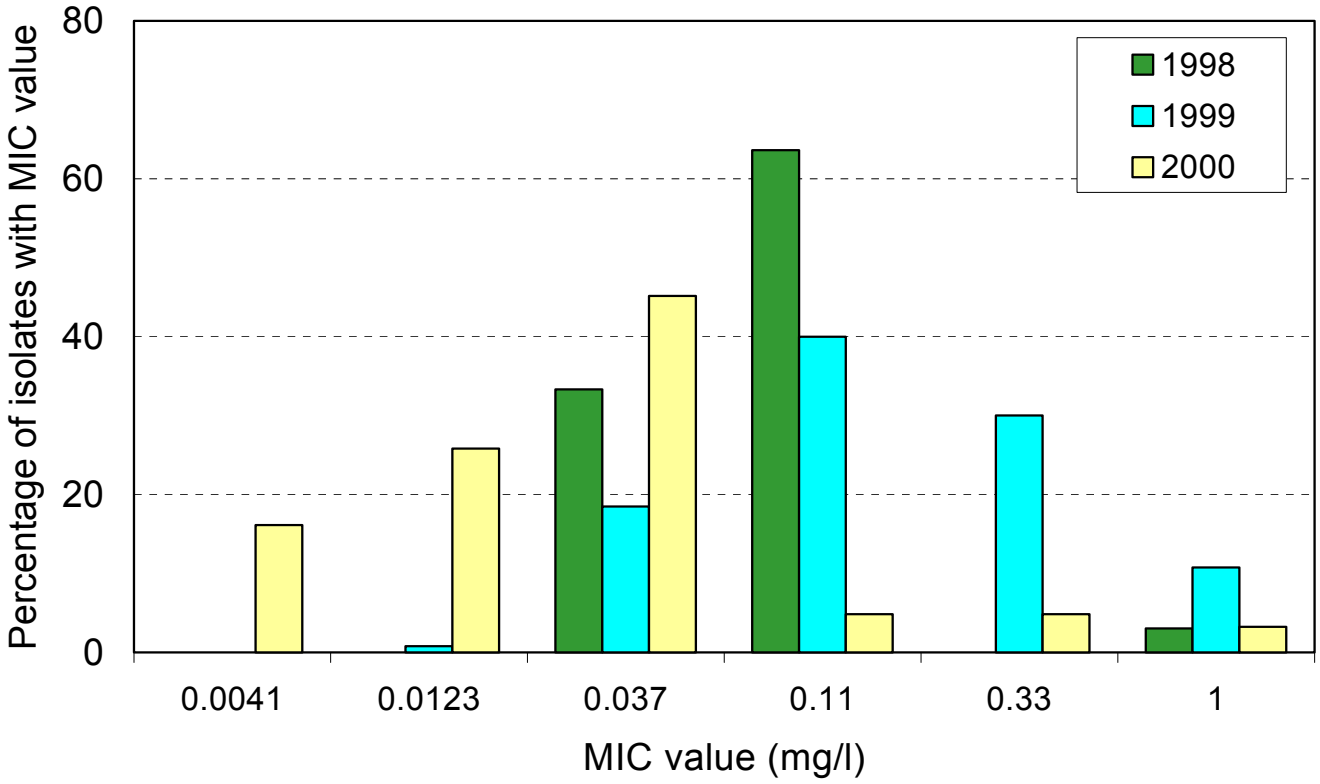
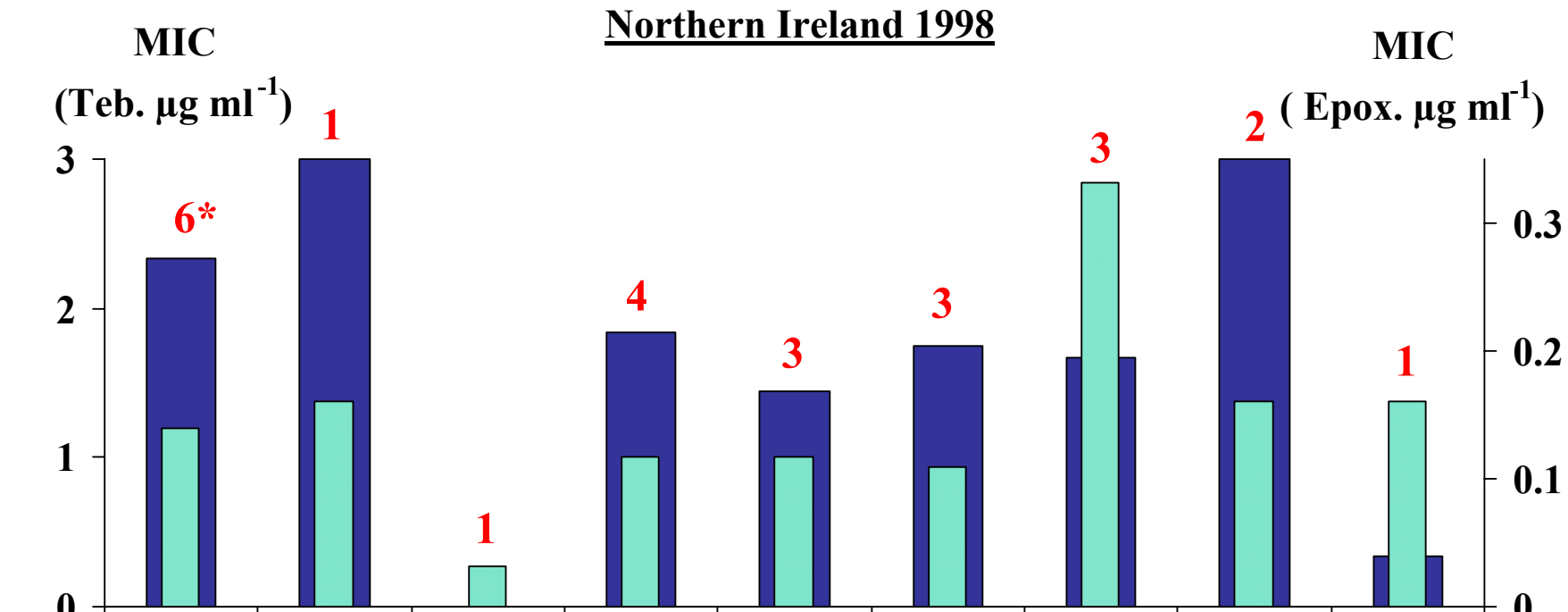
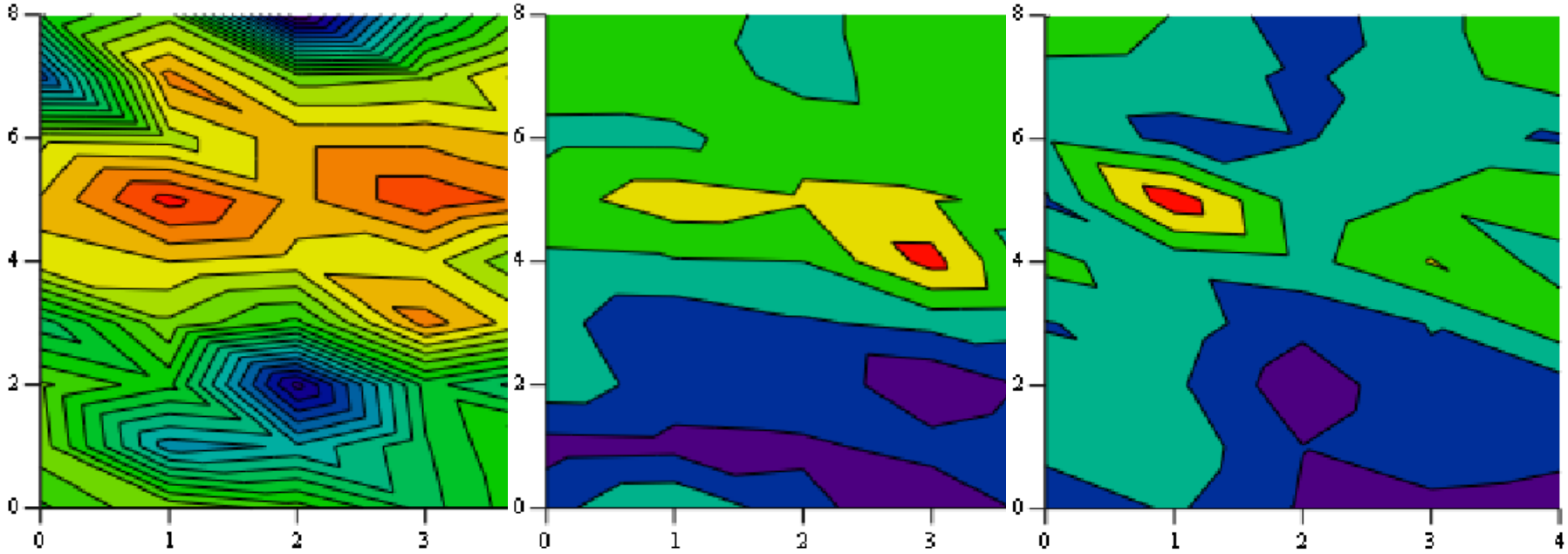


Figure 4 DMI sensitivity of *Mycosphaerella graminicola* from treated crops



	Untreated	Folicur (0.75L/ha)	Folicut (0.5L/ha)	Folicur (0.25L/ha)	Opus (0.5L/ha)	Opus (0.25L/ha)	Amistar (1.0L/ha)	Amistar (0.5L/ha)	Amistar and Opus (0.75L/ha)
Teb	2.33	3	*	1.833	1.444	1.75	1.667	3	0.333
Epox	0.14	0.16	0.032	0.117	0.117	0.109	0.331	0.16	0.16

Figure 5 Contour maps showing sensitivity distributions in *Mycosphaerella graminicola* to three DMI fungicides



Cyproconazole **Flusilazole** **Flutriafol**

Figure 6 Comparison of fungicide effects on *Mycosphaerella graminicola* development in wheat leaves, as monitored by PCR quantification of fungal DNA and visual assessment of symptoms

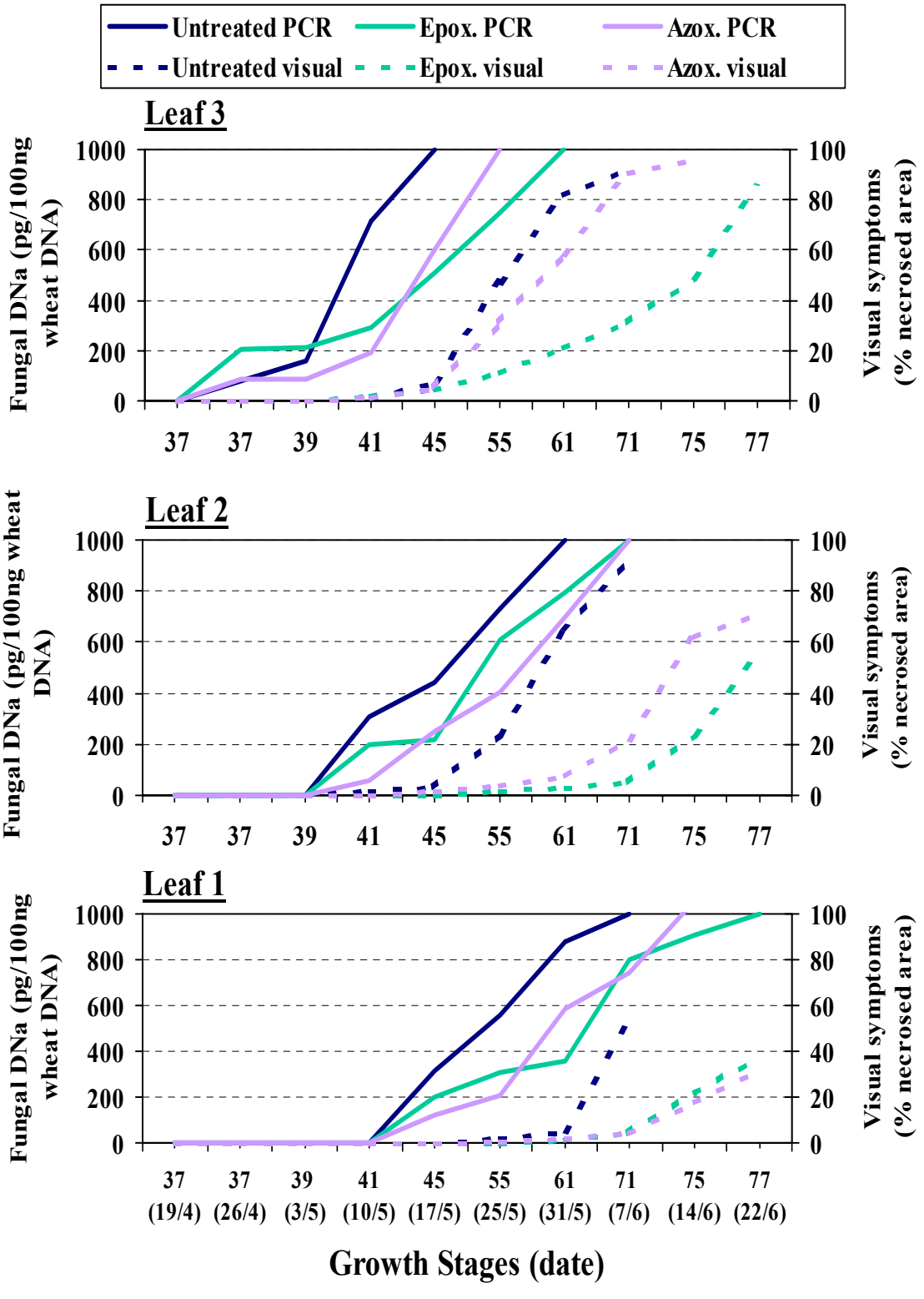


Figure 7: Effect of epoxiconazole (Opus) or tebuconazole (Folicur) both with and without azoxystrobin (Amistar) on *Mycosphaerella gramincola* on winter wheat (Leaf 2)

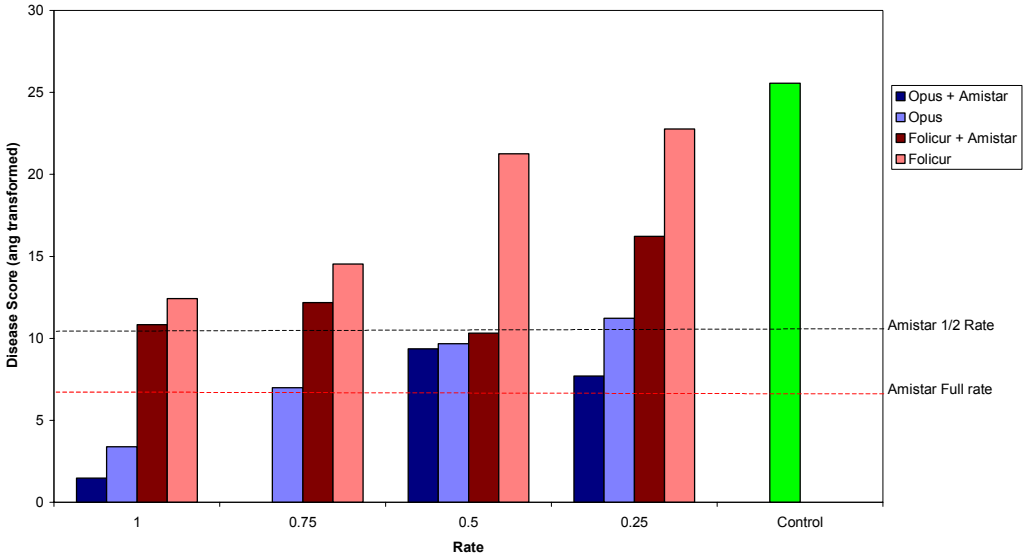


Figure 8: Effect of epoxiconazole (Opus) or tebuconazole (Folicur) with and without azoxystrobin (Amistar) on yield of winter wheat 2000

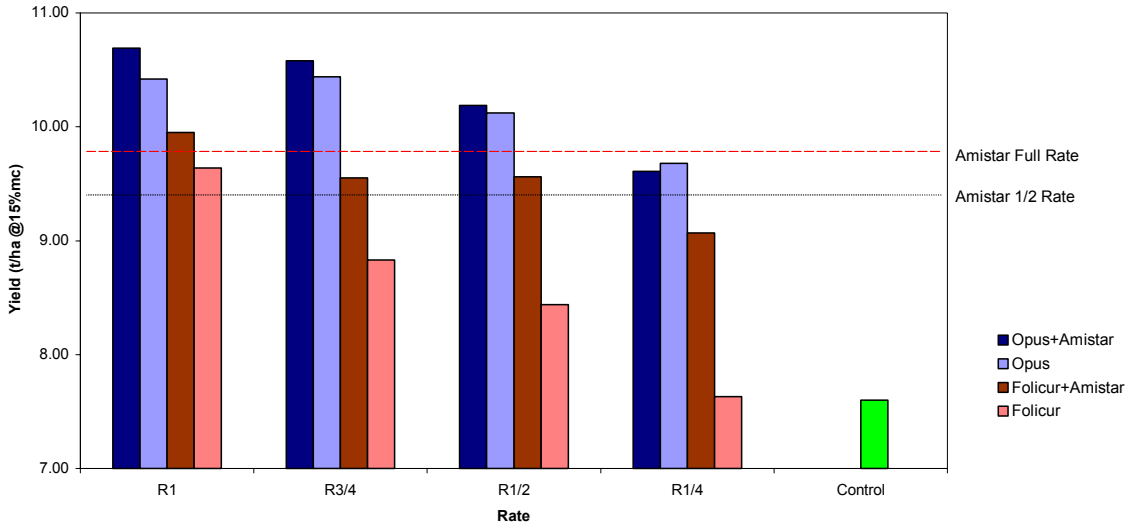
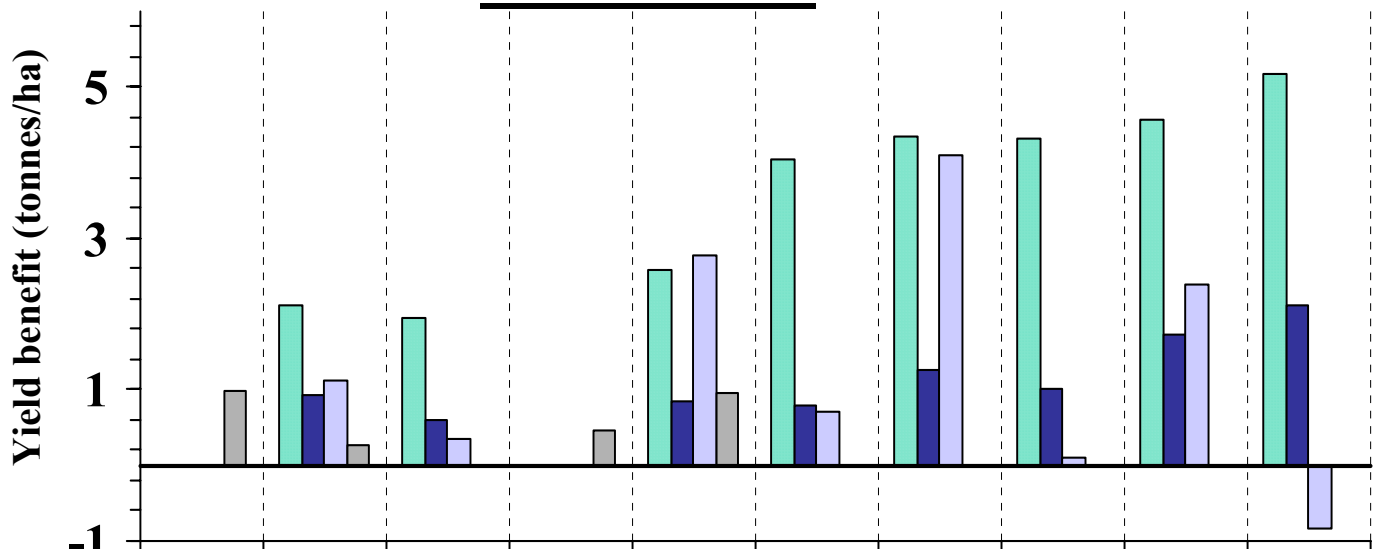


Figure 9 Yield benefit from addition of azoxystrobin to different doses of DMIs for the control of *M. graminicola* in winter wheat



	epox 1.0 / azoxy 1.0	epox 1.0 / azoxy 0.5	epox 0.75 / axozy 0.5	epox 0.5 / azoxy 1.0	epox 0.5 / azoxy 0.5	epox 0.25/ azoxy 0.5	tebu 1.0 / azoxy 0.5	tebu 0.75 / azoxy 0.5	tebu 0.5 / azoxy 0.5	tebu 0.25 / azoxy 0.5
ADAS 1998	-	2.11	1.96	-	2.58	4.04	4.36	4.32	4.57	5.18
ADAS 1999	-	0.94	0.61	-	0.85	0.79	1.27	1	1.74	2.12
Northern Ireland 1999	-	1.12	0.36	-	2.77	0.7	4.09	0.1	2.38	-0.83
LARS 1999	0.99	0.27	-	0.46	0.97	-	-	-	-	-

DISCUSSION

Lesion development and visual symptoms are triggered by some, as yet, poorly understood interaction between *M. graminicola* and its wheat host. Lesion development is, however, a host response and it is clear that whilst some further development of the pathogen can take place, fungicides still exert an effect despite the presence of visual symptoms. Not only is the number of pycnidia in each lesion reduced, but pycnidiospores are not viable, and this will impact very strongly on diseases development within the crop. This may account for the overall good control of disease by DMI fungicides during the cropping season, even though early visual assessments may identify significant amounts of disease following spraying.

Monitoring DMI (Flutriafol) sensitivity in the late 1980s identified a 40–fold difference between the most sensitive isolates (eg. RL2), and ones with reduced sensitivity (eg. S27). Comparison of this UK population distribution with the sensitivity of 80 *M. graminicola* isolates collected in N. America, where no DMIs had been used, showed that all were similar to RL2 (Mapstone & Hollomon, unpublished result, 1989), suggesting that some decline in sensitivity in UK populations had occurred during the early1980s, when DMIs were widely used especially for mildew control. However, no further shift in the UK population seems to have occurred since then, despite the introduction of more effective DMIs, such as tebuconazole, cyproconazole, flusilazole and epoxiconazole. Performance of these fungicides remains good, although yields can benefit from the addition of a strobilurin (QOI; Figure 8).

The non-uniform distribution of DMI sensitivity for *M. graminicola* within a wheat crop, coupled with differences in sensitivity between DMIs which might arise from selection of different mechanisms affecting sensitivity levels, means that many samples must be collected and bioassayed to get a true picture of any response to different anti-resistance strategies. This is in contrast to the situation with the same pathogen and MBC resistance when, after several years of treatment, just one sample from a crop provided an accurate assessment of the resistance situation (Hollomon, unpublished results). A single sample taken from a wheat crop was also sufficient to define the level of strobilurin (QOI) resistance in a wheat crop sprayed three times with a reduced rate of Amistar (azoxystrobin; Fraaije *et al*., 2000).

Applying the geo-statistical analysis of "Kriging" to the data presented in this report suggested that samples should be collected at least 20 metres apart to ensure that they were truly independent of each other. In this case, however, our data set failed to detect any shift in sensitivity following treatment with epoxiconazole, although the number of sample points was halved because of the effectiveness of the fungicide.

Quantitative PCR provides accurate information on the stage of *M. graminicola* infection at the time of fungicide application, and allows evaluation of the relative curative and preventative activity of the different compounds. Since leaf 3 was infected before fungicide sprays were applied, it is clear that azoxystrobin, as well as epoxiconazole, exhibits strong curative action. Azoxystrobin not only slowed the penetration process, but once the pathogen was established within the leaf tissue, the speed of further development was also reduced, although lesion formation was less affected. This strong initial curative activity lasted for at least a week after infection

began, but then gradually weakened until one month later when this curative activity of azoxystrobin was no longer detectable. In contrast epoxiconazole, which controlled Septoria better than azoxystrobin, not only slowed the infection process, but it also delayed subsequent disease progress. These differences between epoxiconazole and azoxystrobin, impact on the spread of disease and especially the extent of necrosis, which is probably an important factor contributing to differences in yield benefits between the two fungicides (Gooding *et al* 2000). One key consequence of these differences in biological activity is that azoxystrobin slows the development of *M. graminicola,* allowing the curative action of expoxiconazole to persist longer. This factor also contributes to the improved disease control and yield benefits achieved by the DMI/azoxystobin mixtures in several field experiments, compared with when the fungicides were used alone.

Preventative activity of all three fungicides (azoxystrobin, chlorothalonil and epoxiconazole) was associated with a reduced number of infections, although complete protection against attack was never achieved. As expected, the preventative activity of epoxiconazole was weaker than for azoxystrobin. In addition to the well understood differences in the biochemical mode of action between DMIs and azoxystrobin, differences in biological activity suggest that as mixture partners they should provide a good anti-resistance strategy. The essentially fungistatic action of azoxystrobin means that strains showing reduced sensitivity and which grow slowly during the infection process, are exposed for longer to the strong curative activity of epoxiconazole . Unfortunately, these experiments provide no information to link the level of Septoria infection with dose rates of the mixture partners, even though an important practical consideration is the need identify the rate of the cheaper DMI required to complement the strobilurin.

Epoxiconazole is the most widely used DMI on wheat (Garthwaite & Thomas, 1998), and when used alone it always gave the best Septoria control of the fungicides used in the two-spray programmes in this project. However, adding azoxystrobin retained green leaf area and lengthened the photosynthetic life of the upper three leaves, and consequently increased yields. As a consequence, the dose rate of epoxiconazole could be reduced to half rate with little yield penalty. These benefits were greater when mixed with the less effective DMI, tebuconazole, and mixtures with azoxystrobin performed almost as well as DMI/epoxiconazole mixtures. Half-rate azoxystrobin was usually sufficient to achieve these benefits (Elcock *et al*., 2000). Although these reduced rate mixtures performed well, there was no evidence that they selected for reduced sensitivity to any of the DMI fungicides tested.

PRACTICAL CONSIDERATIONS

DMI fungicides continue to provide good control of Septoria leaf blotch. But the wide range in sensitivity to these fungicides within field populations of the pathogen, and the cross sensitivity patterns, emphasises that effective anti-resistance strategies should be used if this good performance is to be maintained. This will be especially important if the systemic DMI, fluquinconazole, is used at all widely as a seed treatment for “Take-All” control, since this will prolong exposure of foliar pathogens to selection by DMIs. When choosing mixture partners to reduce selection for resistance, it is crucial that all mixture partners should have activity against target

pathogens throughout the whole period of an epidemic. Because strobilurins slow down *M. graminicola* development, even in applied after infection has occurred, in mixtures they enhance the curative action of DMIs. Consequently, mixtures with strobilurin fungicides not only provide even better disease control and yield benefits than using DMIs alone, but their combined action throughout pathogen development provides a good basis for an anti-resistance strategy. They extent to which DMIs and strobilurins complement each other will be enhanced by introduction of the "second generation" strobilurins, picoxystrobin and pyraclostrobin, since they have even better curative action than azoxystrobin. Several field experiments carried out during this project have emphasised that full rates of the strobilurin partner are not needed to achieve significant yield benefits, but identifying the dose of the DMI partner is more difficult. Using chlorothalonil may add to these benefits, but the purely preventative action of this fungicide means that optimum timing is not always easy to achieve.

Input of accurate information of the levels of infection and DMI sensitivity into DESSAC would enable dose rates of mixture partners to be adjusted to achieve economic levels of control at minimum cost. Use of quantitative PCR provides a good measurement of the amount of disease present, but the DMI sensitivity seen in field populations of *M. graminicola* is far from uniform, and sampling crops to assess accurately the overall level of DMI sensitivity remains a difficulty.

Assessment of visual symptoms two weeks, or less, after spraying is unlikely to provide a meaningful guide to the performance of DMI fungicides, since appearance of necrotic lesions is not necessarily linked to production of viable inoculum and subsequent disease spread. To properly identify any decline in performance of DMI fungicides against *M. graminicola* requires assessment at least six weeks after treatment, when symptoms are the result of new infections occurring well after spraying.

COSTS OF RESISTANCE

Establishing the cost to growers of loosing a fungicide because of resistance is difficult because so many factors are involved, and consequently all the information needed is often not available. However, an attempt was made to assess the cost of resistance to MBC fungicides which, from the mid-1980's, no longer controlled cereal eyespot. The replacement fungicide, prochloraz, was more costly and less effective. The analysis suggested an annual cost to growers at that time of £25m (Locke, 1995). Since MBC resistance in eyespot remains at a high level almost 20 years later, some elements of this cost remain today.

Loss of DMI fungicides because of resistance in *M. graminicola* would be no less costly for growers. Even with the use of generally effective DMI fungicdes, the estimated loss caused by Septoria in 1999 was £24M (Hardwick *et al*., 2000). Again, replacement fungicides are more expensive, and less effective than the best DMI fungicides, such as epoxiconazole. Furthermore, resistance to the alternative strobilurin fungicides has already become a problem in several diseases, including the related *M. fijiiensis* (banana sigatoka disease). Identifying how best to use fungicide mixtures as effective anti-resistance strategies should provide significant cost benefits, if it prolongs the durability of useful DMI fungicides.

FUTURE RESEARCH NEEDS

A key conclusion of this report is that DMI/strobilurin mixtures are a good anti-resistance strategy, and one which should prolong the durability of both fungicide groups. However, used at full rates these mixtures are expensive. There is no evidence that, at least within the fungicide rates used in agricultural practice, that lowering doses encourages DMI resistance in *M. graminicola*. Consequenly, a series of field experiments seeking to link disease levels with fungicide dose rates required to give economic control, should allow the rates of mixture partners to be adjusted to suit individual crop needs, and in this way, minimise costs. Quantitative PCR methods, which were developed with HGCA funding, provide the tools to carry out these experiments in a meaningful way.

ACKNOWLEDGEMENTS

Thanks are due to Professor Harry Parker (Stanford University) for help with the "Kriging" analysis, and to Dr Eric Rohel for contributing to the PCR experiments. We also acknowledge the help of Sheila Kendall, Lisa Black and Patrick Taggart with both the field work and performing the many bioassays carried out during this project.

REFERENCES

Elcock, S.J.; Turner, J.A.; Kendall, S.J; Hollomon, D.W.; Jones, D. Black,L. and Cooke. L.R. (2000) Potential for the development of reduced sensitivity to DMI fungicides in current control practices for *Mycosphaerella graminicola* in winter wheat in the UK. *The BCBP Conference – Pests and diseases 2000*, 407-414.

Fraaije, B.A.; Lovell, D.J.; Rohel, E.A. and Hollomon D.W. (1999) Rapid detection and diagnosis of *Septoria tritici* epidemics in wheat using a polymerase chain reaction/Picogreen assay. *J. Appl. Microbiol.,* **86**, 701-708.

Fraaije, B.A.; Butters, J.A. and Hollomon, D.W. (2000) *In planta* genotyping of *Erysiphe graminis* f.sp. *tritici* isolates for strobilurin resistance using a Fluormetric allele-specific PCR assay. *The BCBP Conference – Pests and Diseases 2000*, 647-654.

Garthwaite, D.G. and Thomas, M.R. (1998) Pesticide Use Survey Report 159; Arable Farm Crops in Great Britain 1998. MAFF and SERAD, 97pp.

Gooding, M.J.; Dimmock, J.P.R.E.; France, J. and Jones, S.A. (2000) Green leaf area decline of wheat flag leaves: the influence of fungicides and relationships with mean grain weight and grain yield. *Ann. Appl. Biol*., **136,** 77-84.

Hardwick, N.V.; Slough, J.E. and Jones, D.R. (2000) Cereal disease control – are fungicides the sole answer? *The BCBP Conference – Pests and Diseases 2000*, 647-654.

Herman, D, and Gisi, U (1994) Cross-resistance among DMI fungicides and sensitivity distributions of *Septoria tritici* populations. . *Brighton Crop Protection Conference – Pests and Diseases,* 487-492.

Leroux, P. and Gredt, M. (2000) Resistance aux fungicides *Mycosphaerella graminicola* (anamorphe *Septoria tritici*), l'agent d'une septoriose du ble. *AFPP- Sixieme conference Internationale sur les Maladies des Plantes*, 795-802

Locke, T. (1995) Fungicide resistance and food production, In: *Proceedings of Japan/UK joint workshop : "Fungicide resistance: a factor limiting quality food production.* Ed. H. Ishii, Japan Science and Technology Agency, Tsukuba, Japan, 34pp.

Joerger, M.C.; Hirata, L.T. and Baxter, M.A, (1992) Research and development of enzyme-linked immunoabsorbent assays for the detection of *Septoria nodorum* and *Septoria tritici*. *Brighton Crop Protection Conference – Pests and Diseases,* 689-695.

Metcalfe, R.J.; Shaw, M.W. and Russell, P.E. (1998) Factors affecting strength of selection for resistance to DMI fungicides in *Septoria tritici*. *Brighton Crop Protection Conference – Pests and Diseases,* 535- 540.

Mittermeier, L.; Dercks, W.; West, S.J.E. and Miller, S.A. (1990) Field results with a diagnostic system for the identification of *Septoria nodorum* and *Septoria tritici*. . *Brighton Crop Protection Conference – Pests and Diseases,* 757-762.

Webster, R. and McBratney, A.B. (1987) Mapping soil fertility at Brooms Barn by simple Kriging. *J. Sci. Food Agr*. **38**, 97-115.

OUTPUT AND TECHNOLOGY TRANSFER

Scientific publications:

Elcock, S.J.; Turner, J.A.; Kendall, S.J; Hollomon, D.W.; Jones, D. Black,L. and Cooke. L.R. (2000) Potential for the development of reduced sensitivity to DMI fungicides in current control practices for *Mycosphaerella graminicola* in winter wheat in the UK. *The BCBP Conference – Pests and diseases 2000*, 407-414.

Kendall, S.J.; Hollomon, D.W. and Selley, A.(1998) Immunodiagnosis as an aid to the timing of fungicide sprays for the control of *Mycosphaerella graminicola* on winter wheat in the UK. . *Brighton Crop Protection Conference – Pests and Diseases, 701-706*.

Others:

Input into the preparation of *"Fungicide Resistance"* produced by FRAG UK (2001)

Presentations:

Talks at various meetings of growers and advisors throughout the country, including HGCA "Roadshows"
Demonstration at Cereals 2000.