PROJECT REPORT No. 139

FUNGICIDE RESISTANCE IN CEREAL PATHOGENS 1991-96: *Rhynchosporium secalis* ON BARLEY; *Erysiphe graminis* ON WHEAT AND BARLEY; *Septoria tritici* ON WHEAT; *Puccinia striiformis* ON WHEAT

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by

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SUMMARY

This report explores resistance questions surrounding fungicides used to control three of the most important cereal diseases in the UK. It involves control of *Rhynchosporium secalis* (leaf blotch) on barley, *Erysiphe graminis* (powdery mildew) on both wheat and barley, and *Septoria* tritici (wheat leaf blotch). A small component also involved yellow rust (*Puccinia striiformis*. The work focused around many field experiments in all parts of the UK during 1991-6, and which have kept abreast of fungicide use strategies as they have developed. Fungicide sensitivity has been monitored by methods appropriate to each disease/fungicide combination, as well as exploring new assay methodologies aimed at increasing accuracy, and throughput of monitoring programmes.

The report identifies benzimidazole resistance in *R. secalis* populations for the first time, and the extent to which its frequency impacts on field performance, especially in wetter regions of the UK. Resistance to some DMIs has also increased in *R. secalis* populations, but field trials clearly show a benefit from mixing existing DMIs with morpholine fungicides. It is suggested that this is one strategy that can be adopted in practice.

Although some decline in morpholine sensitivity in cereal powdery mildew populations may have occurred, especially in 1994 and 1995, changes have been small and unlikely to affect performance.

Selection for reduced sensitivity to DMI fungicides that occurred in *S. tritici* populations prior to 1990 has not continued, and control by a wide range of azole fungicides remains good. Reduced dose rates do not appear to encourage resistance in *S. tritici*, but this may be due to the rapid mobility of the azole (Flutriafol) used which may allow survival of a reservoir of sensitive individuals in the basal, unprotected, parts of leaves.

The appearance of many new fungicides with systemic protectant action may alter how fungicides are used. How to incorporate early applications of persistent products into effective anti-resistance strategies, and preserve the usefulness of existing, broad-spectrum products, should be a key element of any future independent research programme. Mixtures of fungicides with different modes of action are likely to remain the main anti-resistance strategy, and research resources need to be focused on optimising these strategies.

Novel monitoring methods can improve the accuracy and number of field isolates that can be tested. Lack of good quantification limits the value of dot blot methods, but developments in DNA diagnostic technologies should provide robust, micro-titre plate assays, which overcome these difficulties.

INTRODUCTION

Fungicides are important components in the management of many cereal diseases. Until recently the choice of fungicides available to growers was limited, with just four different systemic groups and one non-systemic protectant, chlorothalonil, being used to control foliar or stem-base cereal diseases. The properties of these fungicides were often exploited in mixtures, which also play a significant role as anti-resistance strategies. For both growers and manufacturers alike, fungicide resistance represents a waste of resources, which can only be

dealt with through more costly, and often less effective, substitutes. It is difficult to calculate the full extent of these costs to growers, but a recent analysis of benzimidazole (MBC) resistance in cereal eyespot (*Pseudocercosporella herpotrichoides*), put losses caused by resistance above £50 m over the past decade (Locke, 1996).

During the 1980s, HGCA funded a series of studies which established the base-line sensitivity for most of the important cereal diseases (reference). UK-wide monitoring of *Rhynchosporium secalis* populations provided early warning of resistance to sterol 14α demethylase inhibiting fungicides (DMIs), such as triadimenol, and provided a framework to advise growers to use DMI/benzimidazole mixtures as an anti-resistance strategy. It was the need to re-evaluate this strategy in the light of the detection of benzimidazole-resistant *R. secalis* strains for the first time in 1991, that prompted some of the research described in this report.

For a number of reasons growers have sought to reduce fungicide dose rates wherever possible. The consequences of this on selection for resistance is unknown, and so our programme examined this question using both *Erysiphe graminis* (cereal powdery mildew), and *Septoria tritici*, the cause of wheat leaf blotch. *S. tritici* is well suited to this work for a variety of reasons. Although extremely variable, most of this variation will be found within a single experimental site (McDonald and Martinez, 1991), so these populations will be representative of the surrounding farming district. New populations are established each autumn from ascospore infections (Shaw and Royle, 1989), so that the previous history of fungicide use at any site will have little influence on the *S. tritici* population within it.

Studies outlined here require monitoring methods capable of rapidly and accurately identifying resistance in many thousands of samples. Improvements in bioassay through use of micro-titre plates have been explored, whilst recent innovations surrounding the Polymerase Chain Reaction (PCR) were harnessed to identify frequency changes in benzimidazole resistance in *R. secalis* populations.

OBJECTIVES

1. To monitor the sensitivity of cereal mildew populations to morpholine fungicides, *P. striiformis* to triazoles. Particular attention was paid to charting the spread of benzimidazole resistance in *R. secalis*.

2. Evaluate strategies to combat resistance in *R. secalis*.

3. Examine the effects of reduced fungicide rates on selection for resistance in both *S. tritici* and cereal powdery mildew populations.

4. Evaluate the usefulness of biochemically and microbiologically based methods to rapidly monitor fungicide sensitivity.

Table 1. **Programme schedule**

Objective	Time Scale	Collaborator
1. Monitoring		
R. secalis	1991-6	LARS, DANI, ADAS, IGER
S. tritici	1992-3 1996	DANI LARS
P. striiformis	1991-3	NIAB
Cereal powdery mildews	1991-6	SAC
2. Anti-resistance strategies		
R. secalis	1991-6	LARS, DANI, ADAS
3. Dose rate		
S. tritici	1991-5	RU
barley powdery mildew	1991-5	SAC
wheat powdery mildew	1991-6	SAC
4. Novel monitoring methods		
S. tritici	1991-2	RU
Biochemical	1993-6	LARS, ADAS, DANI

ADAS Agricultural Development and Advisory Service

DANI Department of Agriculture, N. Ireland

IGER Institute of Grassland and Environmental Research

LARS Long Ashton Research Station

NIAB National Institute of Agricultural Botany

RU Reading University

SAC Scottish Agricultural Colleges

METHODS

R. secalis

1. Monitoring

Conventional bioassays on fungicide amended medium (Czapek Dox plus Mycological Peptone; CDM; Kendall *et al* 1994) formed the cornerstone of the survey work. Isolations were made from surface sterilized lesions onto CDM containing chloramphenicol (100 μg ml^{-1}) to prevent bacterial contamination. Established isolates were tested without further purification, although in some cases single spore isolates were established for further testing. Sensitivity was determined as Minimum Inhibitory Concentration (MIC).

2. DNA probe technology

Benzimidazole resistance is the only example at present where the mechanism is understood at a molecular level. Single DNA base changes causing a limited number of amino acid replacements in the target β-tubulin are correlated with resistance in many pathogens, including *R. secalis*. Modern DNA diagnostic technologies involving the Polymerase Chain Reaction (PCR) allow detection of single base changes, and this technology has been exploited in an effort to develop a rapid and accurate method to detect benzimidazole resistance in *R. secalis*. Precise details of the method that has been developed have already been published (Wheeler *et al.*, 1995) but a key feature involves production of the PCR template DNA simply by boiling leaf material. PCR was used to amplify a fragment of the beta tubulin gene which contained the region where base-pair changes occur, and this fragment bound to a nylon membrane.

Alterations were detected with specific biotin labelled, oligonucleotide probes, coupled with streptavidin-alkaline phosphatase ELISA technology to provide a blue colour. Careful control of the temperature used to wash membranes and distinguish probes that matched exactly the amplified DNA sequence, from probes that had a single base pair mis-match.

3. Field experiments

At least five field experiments were carried out each year and it is not practical to describe each one in detail. The experiments centred around the evaluation of fungicide mixtures as anti-resistance strategies, and were carried out in both N. Ireland and the western part of England. All relied on natural infection. Fungicide applications varied from GS 31-33 at sites in S.W. England, to GS 65-75 in N. Ireland. Experiments were carried out on both winter and spring barley, whilst at LARS and Rothamsted autumn sowing of the susceptible Spring variety, Chariot, normally ensured heavy infections. Except for some early Rhynchosporium trials in 1995 on winter barley, the exceptionally hot weather during the summer of that year limited disease development.

S. tritici

1. Monitoring

Infected leaf material was placed on moist filter paper so that pycnidia were induced to release cirrhi. These were streaked on to Potato Dextrose Agar (PDA) containing streptomycin sulphate (100 μg ml^{-1}) and single pycnidiospore cultures isolated. Sensitivity was tested on PDA amended with fungicides, and assessed as MIC values.

2. Microtitre plate assay of *S. tritici*

On many culture media *S. tritici* has a yeast-like growth which lends itself to measurement in micro-titre plates. Full details of the method that was developed to test the sensitivity of isolates from different field plots to flutriafol, is given in Pijls *et al.* (1994). An essential feature of this method was the release of pycnidiospores from individual cirrhi into sterile water, and use of these spore suspensions to inoculate micro-titre plate wells containing Czapek-Dox liquid media and up to 12 different flutriafol concentrations. Success also depended on incubating these plates with constant shaking under conditions that ensured little or no condensation. Dose response curves were fitted to absorbance data which measured growth, and sensitivity levels [ED_{50}s] correlated well with *in vivo* tests on wheat seedlings. Two standard isolates of known flutriafol sensitivity were included in every test; very few tests had to be abandoned because of bacterial contamination.

3. Field experiments

These were conducted each year at two sites some 20 km apart (Sonning, Reading, and Jealotts' Hill, Bracknell) and full details are given in Shaw and Pijls (1994). Two winter wheat cultivars, Riband (susceptible) and Mercia (moderately resistant) were used and four different fungicide treatments reflecting agricultural practice. Apart from the no fungicide control, other treatments included full rate of flutriafol, one quarter rate and flutriafol/chlorothalonil mixture (Impact Excell). Plots were sprayed at GS 37 and sampled no more than six weeks later to ensure that a second generation of pycnidia which had not been exposed to fungicide, was not sampled.

Powdery mildews

1. Monitoring

Full details of the assay methods are given in Zziwa and Burnett (1994). Briefly, isolates were collected from field sites and maintained on detached leaves in isolation until tested. Seedlings of wheat and barley were sprayed with up to 14 different doses of each morpholine fungicide as a formulated product (Fenpropimorph = "CORBEL"; Fenpropidin = "PATROL"; Tridemorph = "CALIXIN"). When dry seedlings were dusted with conidia and kept in a greenhouse for 7 days before assessing mildew levels as percentage leaf infected. ED_{50} values were calculated by regression analysis. Treated plants were kept as separate as practical to prevent cross-contamination of treatments due to the high vapour action of especially fenpropimorph.

2. Field experiments

The cereal mildew experiments were carried out in Scotland, and explored the use of fenpropimorph : propiconazole mixtures both on barley and wheat. Generally, two sprays were applied, and samples taken for monitoring fenpropimorph sensitivity before spraying, and three weeks after each spraying. Table 2 gives the protocol of one such experiment.

Table 2. Fungicide programmes evaluated in 1992 field experiment. Bush Cereal Centre.

Treatment	First application		Second application	
U	nil		nil	
A	fenpropimorph	1.0*	fenpropimorph	1.0
B	fenpropimorph	0.5	fenpropimorph	0.5
C	fenpropimorph	0.25	fenpropimorph	0.25
D	fenpropimorph	1.0	fenpropimorph	1.0
	+ propiconzole	0.5	+ propiconzole	0.5
E	fenpropimorph	0.5	fenpropimorph	0.5
	+ propiconazole	0.25	+ propiconazole	0.25
F	fenpropimorph	0.25	fenpropimorph	0.25
	+ propiconazole	0.125	+ propiconzole	0.125
G	nil		fenpropimorph	0.25
			+ propiconazole	0.125

* dose rates as a proportion of the full commercial dose of the products used: full commercial doses for the products used were as follows:

Active ingredient	Product	g AI/ha
fenpropimorph	Corbel	750
propiconazole	Tilt 250 EC	125

All fungicides were applied using a tractor mounted Allman hydraulic sprayer with standard flat fan nozzles in 270 l/ha of water at a pressure of 2 bars.

Yellow rust

1. Monitoring

Isolates of yellow rust (*Puccinia striiformis*) were collected throughout the UK, and stored as freeze-dried uredospores in sealed ampoules until tested for their sensitivity to fenpropimorph or fenpropidin. Seedlings of a susceptible wheat cultivar (Sappo or Vuka) were sprayed when the first leaf had fully expanded with fenpropimorph (= CORBEL) at 187 mg ai. l^{-1} (= 1/20th field rate), or fenpropidin (PATROL) at 375 mg ai. l^{-1} (1/10th field rate). Two weeks later the percentage leaf area infected was assessed and the results used to calculate a "I" value which provided a measure of sensitivity.

$$I = P_{tf} / P_{to} - P_{sf} / P_{so}$$

Where P_t = percentage infection of test isolate

P_s = percentage infection of standard isolate

f = fungicide treated

o = untreated

Positive values of I indicated higher infection levels than the standard isolate ie. lower sensitivity.

RESULTS

Rhynchosporium secalis: **Barley leaf blotch**

Changes in carbendazim sensitivity in *R. secalis*

Before 1991 no stable carbendazim resistant isolates were detected anywhere in the UK, despite testing more than 4,000 isolates. But in that year, highly resistant (HR; MIC = 25 $\mu g\ ml^{-1}$ or above) and moderately resistant (MR; MIC = 10 $\mu g\ ml^{-1}$) strains were detected at several places in the UK. Results from Northern Ireland (Figure 1) reflect what happened in other parts of the UK. Although the frequency of carbendazim resistant strains increased in 1991 and 1992, it has not changed since. The actual frequency of resistant strains can differ substantially between sites (Table 3), emphasising the erratic spread of carbendazim resistance within Rhynchosporium populations. Further evidence of geographical differences in the response of *R. secalis* to selection with benzimidazole fungicides was provided by detailed monitoring of field trials carried out to evaluate different anti-resistance strategies. At Mahee Island in Co. Down in 1994 two applications of carbendazim (Derosal) increased the frequency of benzimidazole resistant strains four-fold (Figure 2), whereas at Rothamsted the following year the same treatments hardly increased the frequency of resistant strains at all (Table 4). At both sites the initial frequency of resistant strains in those plots subsequently sprayed, was around 10%. 110 barley crops were sampled in England and Wales in 1992 and 1993, and 759 isolates were tested. The frequency of carbendazim resistance was erratic ranging from 3% to 47% in some crops. Regional differences were again apparent, with resistant isolates more common in wetter, western regions.

In general, cultivar had no effect on the frequency of carbendazim resistance, but a randomised survey of 74 crops in England and Wales in 1993, in which 639 isolates were tested, identified the cultivar Pipkin, with a significantly lower level of resistant isolates than all other cultivars surveyed (Table 5). A limited amount of work carried out subsequently supported this observation. Surprisingly, throughout all these monitoring studies no link was found between the use of benzimidazole fungicides, and carbendazim resistance.

Figure 1. **Sensitivity of N. Ireland *Rhynchosporium secalis* isolates to carbendazim, 1990-1996**

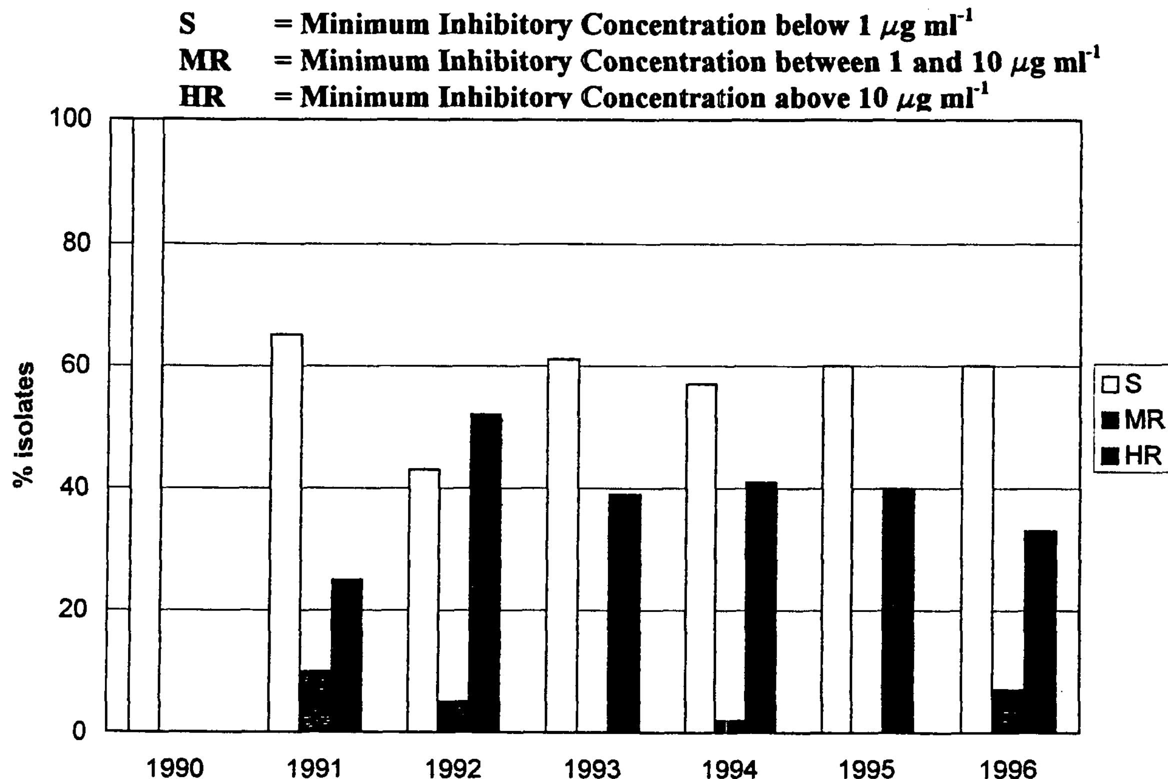


Figure 2. **Effect of two applications of carbendazim on the frequency of resistant strains in two *R. secalis* populations at Mahee Island, Co. Down in 1994**

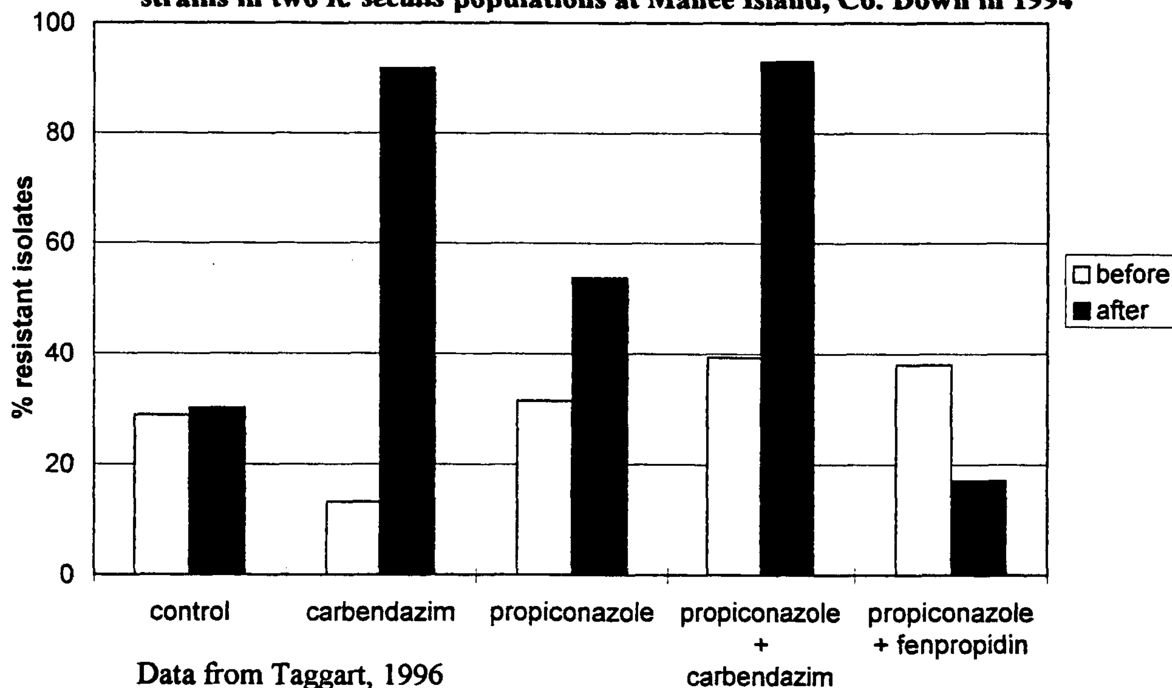


Figure 3. Benzimidazole and N-Phenylcarbamate fungicides

Common name	Chemical name	Manufacturer trade name	Structure
Benomyl	Methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate	Du Pont Benlate® Chinoin Fundazol®	$CO-NH-C_4H_9$; N; N; $NH-COOCH_3$
Carbendazim	Methyl benzimidazole-2-yl carbamate (MBC)	Du Pont Delsene® BASF Bavistin® Hoechst Derosal®	N; N H; $NH-COOCH_3$
Fuberidazole	2-2'-furyl)-1H-benzimidazole	Bayer Neo-Voronit®	N; N H; O
Thiabendazole	2-(4'-thoazolyl)-benzimidazole	Merck Mertect®	N; N H; N; S
Thiophanatemethyl	Dimethyl 4,4'-o-phenylene bis (3-thioallophanate)	Nippon Soda Cercobin-M® Topsin-M®	$NH-C(=S)-NH-COOCH_3$; $NH-C(=S)-NH-COOCH_3$
Diethofencarb	**3,-4-diethoxyophenyl carbamate**	**Sumitomo Sumico R**	C_2H_5O; C_2H_5O; $NHCOOCH(CH_3)_2$

Table 3. **Percentage frequency of highly carbendazim resistant strains of *Rhynchosporium secalis* in different parts of the UK in 1995.**

Site	Cultivar	% Frequency
N. Ireland	Various	40
Ross-on-Wye	Halcyon	20
Rothamsted	Chariot	10
Long Ashton	Chariot	9

Table 4. **Frequency of carbendazim resistant strains following treatment with carbendazim (Bavistin) at Rothamsted in 1995.**

	% resistant strains		Total number of strains tested
	MR	HR	
Before first treatment	4	8	28
After first treatment	3	6	37
After second treatment	6	10	35

MR = Moderately resistant

HR = Highly resistant

Data from Pask (1995). Differences between treatments are not significant

Table 5. Incidence of carbendazim resistance in *R. secalis* by crop and isolate according to cultivar.

Winter barley cultivar	CROP		ISOLATE	
	Number sampled	% with resistant isolates present	Number tested	% resistant to carbendazim
Marinka	6	83.3	53	47.2
Pastoral	14	64.9	114	36.0
Fighter	13	46.2	121	13.4
Halcyon	4	50.0	40	10.0
Puffin	11	36.4	96	9.4
Pipkin	14	14.3	113	2.7
Other cultivars	12	33.3	102	7.8
All cultivars	74	45.9	639	16.6

Data from Phillips and Locke (1994)

Negative cross resistance

An interesting aspect of benzimidazole resistance is its association with increased sensitivity to N-phenylcarbamate and related fungicides (Figure 3). This offers scope to manage benzimidazole resistance using mixtures of phenylcarbamate and benzimidazole fungicides. A strategy based on this mixture has been used to control grey mould (*Botrytis cinerea*) on grapes and lettuce, and to control *Colletotrichum* spp. on strawberries, especially in Japan. Whilst these strategies have largely been successful, especially in Japan, any benefits were nullified in some regions of France because of doubly-resistant strains. We have, therefore looked at the sensitivity of *R. secalis* to both carbendazim and the N-phenylcarbamate fungicide diethofencarb, and can confirm that the strong negative cross-resistance between these two fungicide groups also occurs in this pathogen (Table 6). In N. Ireland, where 43% of isolates tested were carbendazim resistant, almost all (38% of the total) showed negative cross-resistance. Five percent (of the total) were also resistant to both fungicides. Evidence of negative cross-resistance was obtained from *R. secalis* populations from both Long Ashton and Rothamsted, although at these sites over half the carbendazim resistant strains were also resistant to diethofencarb.

Table 6. **Cross-sensitivity between diethofencarb and carbendazim in natural populations of *Rhynchosporium secalis.***

Location	Years	No. of isolates tested		% Total isolates			
			Carbendazim....	S	MR	HR	HR
			Diethofencarb...	R	R	S	R
N.Ireland	1990-5	204		57	0	38	5
Long Ashton Rothamsted	1995	160		90	2	3	5

S = Sensitive; MR = Moderately Resistant; HR = Highly Resistant; R = Resistant

Table 7. **Cross-resistance between DMI and carbendazim fungicides at two sites in England in 1992.**

Fungicide and concentration ($\mu g\ ml^{-1}$)		Number	%
triadimenol	0.8	43	12.22
	3.2	8	2.27
	12.8	38	10.80
	51.2	200	56.82
	51.2	63	17.90
propiconazole	0.008	2	0.57
	0.04	19	5.40
	0.2	57	16.19
	1	174	49.43
	5	91	25.85
	5	9	2.56
prochloraz	0.0016	8	2.27
	0.008	10	2.28
	0.04	70	19.89
	0.2	235	66.76
	1	25	7.10
	1	4	1.14
tebuconazole	0.016	13	3.69
	0.08	14	3.98
	0.4	53	15.06
	2	220	62.50
	10	48	13.64
	10	4	1.14
carbendazim	1	345	99.13
	5	7	0.87
total number of isolates		352	

Table 8. Control of *Rhynchosporium secalis* by carbendazim.

Site	Year	Frequency of carbendazim resistant strains		Rhynchosporium levels		% disease control
		Before Treatment	After treatment	Untreated	Treated	
Strangford	'93	95	85	25.6	31.7	Significant increase
	'94	55	85	17.5	14.1	15 NS
Mahee Island	'94	30	90	7.8	5.2	15NS
Newtonards	'94	25	88	10.9	10.2	8 NS
Portaferry	'93	10	42	24.8	8.3	66***
Long Ashton	'95	10	18	11.4	7.1	40***
Rothamsted	'95	18	18	5.9	1.4	75***
Goathurst (Somerset)	'94	25	36	7.9	8.6	Increase NS
Beckbury (Shropshire)	'94	0	1	2.1	1.2	43***

Disease levels are the mean of the % leaf area infected for leaves 1 (Flag leaf) and 2 assessed one month after the final treatment.
*** Significant (P < 0.001)

Cross Resistance - DMIs

Isolates were not only tested for their sensitivity to carbendazim and diethofencarb, but also to the DMI fungicide triadimenol. (In some cases sensitivity to propiconazole was also assayed.) Although triadimenol resistance was widespread in *R. secalis* populations from England and Wales when this project began in 1990, this was not the case in N. Ireland (Figure 4). By 1992, however, populations in Ulster were no different from those elsewhere; two distinct phenotypes with different triadimenol sensitivities (RF = >100) were clearly evident. Cross-resistance exists between triadimenol and propiconazole, but unlike the situation with triadimenol the sensitivity distribution for propiconazole is unimodal.

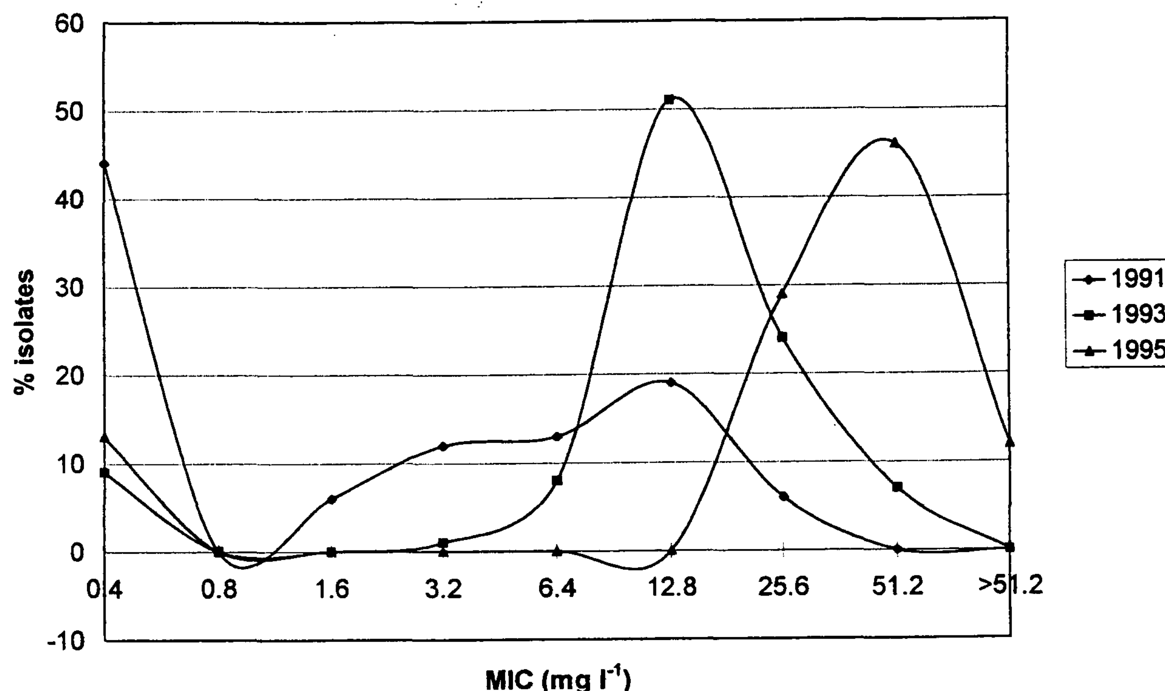
Although the mechanism of action of benzimidazole and DMI fungicides are not the same, there was surprisingly, a strong link between carbendazim and triadimenol sensitive isolates collected in N. Ireland (X^2 test p = <0.001). This link was not observed elsewhere in the UK. In one survey ccarried out in 1994 at two sites in England the majority (80%) of isolates were MBC sensitive but DMI (triadimenol) resistant. Only 2% of isolates were sensitive to both fungicides (Table 7).

Effect of carbendazim resistance on performance

a) *As a single product*

Results from nine field trials were available to evaluate any link between carbendazim resistance and performance (Table 8). Selection for resistance clearly affected performance of carbendazim, and where carbendazim treatments increased the frequency of resistance strains in Northern Ireland to 85% or more, control of Rhynchosporium was poor. Indeed, at the Strangford site in 1993, disease levels were significantly increased by treatment with carbendazim. At Goathurst in Somerset carbendazim failed to control even low levels of *Rhynchosporium* despite the fact that two thirds of the isolates tested were carbendazim sensitive. Where selection was less effective, for whatever reason, control was often acceptable, and at Long Ashton in 1995 carbendazim was the best fungicide treatment used. Performance was not correlated with either the initial frequency of carbendazim resistance or disease levels prior to treatment.

Figure 4. Sensitivity of N. Ireland *Rhynchosporium secalis* isolates to triadimenol (1990-95).



Performance of fungicide mixtures

a) *Disease control*

Field trials carried out in this project all evaluated not only the performance of carbendazim, but also DMI/carbendazim and DMI morpholine mixtures. Some of these results are given in Table 9. With the exception of one trial at Long Ashton in 1995, DMI/morpholine mixtures gave good control of Rhynchosporium, regardless of the frequency of carbendazim or DMI resistance. Performance of DMI/carbendazim mixtures was also good. Only at Strangford in 1993, when carbendazim treatment actually increased Rhynchosporium infection levels, did Hispor (carbendazim + propiconazole) not provide any control of Rhynchosporium. An experimental mixture of propiconazole plus cyprodinil was also effective. Despite the decline

in DMI sensitivity observed in all parts of the UK, propiconazole (Tilt) was no worse than any of the mixture treatments.

Table 9. Performance of DMI mixtures against *Rhynchosporium secalis* at several trial sites, 1993-1995.

1993 Strangford Carbendazim resistant

	% leaf area infected 4 weeks after treatment	
	Leaf 1	Leaf 2
Control	31.7 a	19.6 bc
Derosal	29.7 a	33.7 a
Tilt	8.1 b	9.2 d
Hispor	15.1 b	25.4 ab
Legend	7.2 b	10.1 cd

1994 Mahee Island Carbendazim resistant

	% leaf area infected 4 weeks after treatment		
	Leaf 1	Leaf 2	Leaf 3
Control	2.8 a	12.7 a	16.9 a
Derosal	1.1 a	9.2 b	13.5 ab
Tilt	0.8 a	6.5 bc	10.9 bc
Hispor	0.4 a	5.6 c	10.3 bc
Legend	0.4 a	8.0 bc	8.8

Table 9 (continued)

1995 Long Ashton Carbendazim sensitive

	% leaf area infected 4 weeks after treatment	
	Leaf 1	Leaf 2
Control	6.5 a	15.9 a
Bavistin	7.5 a	6.6 c
Hispor	8.5 a	13.0 b
Tilt Turbo	9.5 a	25.0 d

1995 Holsworthy (Devon) and Bobbington, Staffs. (Mean of two sites)

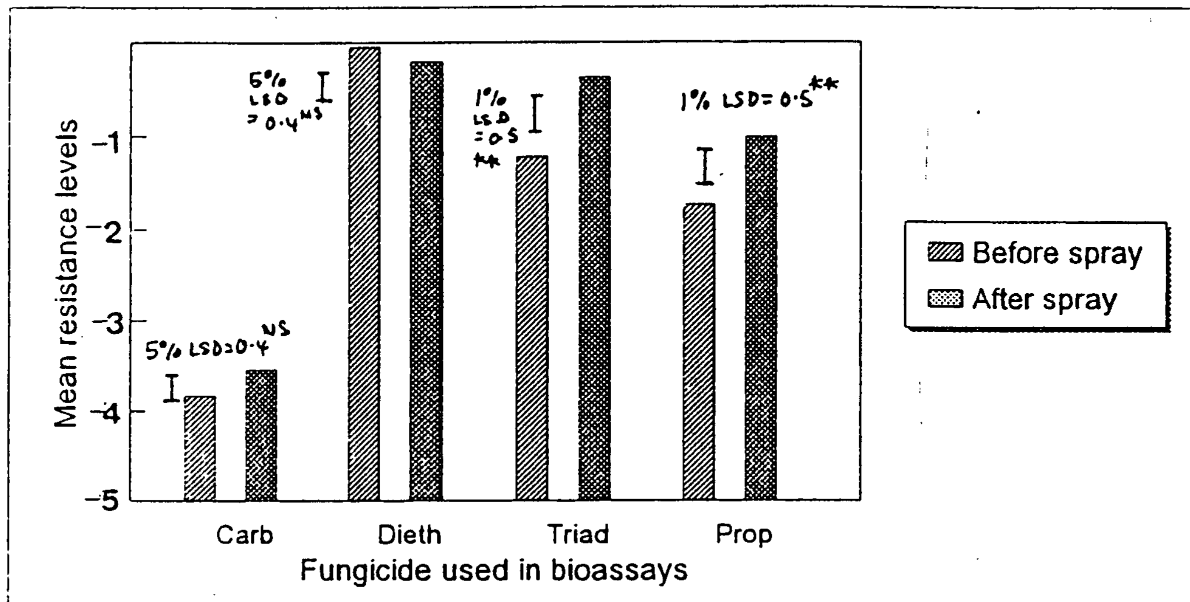
	% leaf area infected 6 weeks after treatment (GS69)		
	Leaf 2	Leaf 3	Leaf 4
Control	1.0 a	6.7 a	28.4 a
Sportak Delta	0.4 b	3.5 b	12.6 b
Glint	0.4 b	2.6 b	10.5 b
Sprint	0.4 b	2.2 b	8.2 bc
Fusion	0.2 b	1.9 bc	7.0 bc
Opus Team	0.1 b	0.8 c	6.4 c
SL 560A	0.3 b	1.9 bc	9.9 bc

Numbers followed by the same letter in each column do not differ significantly ($p > 0.05$)

All fungicides and mixtures were used at recommended rates.

Derosal = Carbendazim
Bavistin = Carbendazim
Tilt = Propiconazole
Hispor = Propiconazole + Carbendazim
Glint = Propiconazole + Fenpropimorph
Sprint = Prochloraz + Fenpropimorph
Fusion = Flusilazole + Tridemorph
Opus Team = Epoxiconazole + Fenpropimorph
SL 560A = Propiconazole + Cyprodinil (Experimental mixture)

Figure 5. Impact of fungicide mixtures on selection for resistance in *Rhynchosporium secalis*.



Differences existing between *R. secalis* isolates sampled either 1 week before plot treatment or during a period between 4 to 8 weeks after plot treatment in the levels of resistance to 4 fungicides used in the bioassay assessments. Mean resistance levels for isolates sampled at LARS and RES is shown. Resistance level 1-5 indicates increasing MIC level. to 4 fungicides . .

b) *Selection for resistance*

Although attempts were made to collect and assay ten isolates from each plot before and after fungicidal treatment, considerably fewer isolates were obtained in many cases. The small number of isolates assayed from each plot required some pooling of data. Analysis of data from Long Ashton and Rothamsted trials was achieved using an unbalanced design in Genstat after first coding the sensitivity of each isolate on a scale 1-5 based on its MIC value (5 = highest resistance). This analysis showed no significant impact ($p = 0.05$) of mixtures on the level of carbendazim (and diethofencarb) resistance (Figure 5). (A decrease in the frequency of carbendazim resistance following treatment with a DMI/morpholine mixture was almost significant at the LARS site, and a larger data set may have confirmed this). The analysis of data from the 160 isolates did, however, show that sensitivity to both propiconazole and triadimenol declined at both sites, although this change occurred on untreated plots as well as treated ones.

Analysis of data from N. Ireland was carried out after pooling assay results from four sites. In this case, the effect of fungicide treatment on the frequency of carbendazim resistance was identified as the probability of detecting a resistant isolate after treatment, compared with the probability before treatment. Analysis of variance was used to assign significance to these probabilities. Table10 shows the format of this analysis. It confirms that significant differences in the frequency of carbendazim resistant isolates existed between the different sites, and their frequency increased with time. A key feature of the results is that treatment with carbendazim, either alone or in a mixture, increased the frequency of resistant strains. Propiconazole, and the propiconazole/fenpropimorph mixture decreased the probability of encountering carbendazim resistant strains but, as at Long Ashton, these changes were not significant. A similar analysis of triadimenol sensitivity was not carried out.

Detection of benzimidazole resistance using allele specific oligonucleotide probes

Single spore isolates from infected control plots at LARS were assessed for benzimidazole resistance using either bioassay, or a PCR diagnostic. Some isolates were tested by both methods, others were not. The probes used detected either a sensitive allele (Glutamate 198 GAG) or the most common resistance allele (Glycine 198 GGG). The majority of isolates were clearly sensitive (Table 11) although use of allele specific probes identified three times as many resistant isolates as did the bioassay method. This comparison was extended and 159 isolates were all tested by both methods although the PCR test was carried out several months after the bioassay, and after isolates had been subcultured at least twice on fungicide free CDM medium. Again the majority of isolates were benzimidazole sensitive. The overall correlation between the two methods was good, and 88% of isolates were classified the same by both methods (Table 12). However, the PCR diagnostic detected only half the number of isolates judged resistant by bioassay. Three isolates classified sensitive by bioassay were classified as resistant using the allele specific oligonucleotide probe.

All these PCR diagnostic tests were carried out by LARS staff familiar with the technology involved. In a separate series of tests, the diagnostic was evaluated alongside conventional bioassays in N. Ireland using PCR primers and probes supplied from LARS. Twenty-two isolates (not single spore isolates) were obtained from half lesions and bioassayed; the other half of each lesion was boiled in water to provide a DNA template for PCR. Half the isolates bioassayed grew at a single discriminatory dose of carbendazim (10 $\mu g\ ml^{-1}$) and were classified as resistant. Examination of the final dot blots was not easy because of high

backgrounds, possibly because a nitrocellulose rather than charged nylon membrane was used. Only six lesions produced a positive resistant reaction following PCR and hybridization with probes corresponding to sensitive (198; GAG), resistant (198; GGG) or resistant (200; TAC). These were all with the GGG resistant probe.

Table 10. Carbendazim sensitivity shifts at four sites in N. Ireland. From Taggart, 1996.

Factor	Parameter	Standard error	Significance
constant	1.854	0.333	***
SITE P'ferry 93	-4.321	0.400	***
SITE S'ford 94	-1.520	0.320	***
SITE N'ards 94	-2.674	0.326	***
SITE Mahee Isl. 94	-2.674	0.326	***
FUNG Carb	0.098	0.323	ns
FUNG Prop	-0.033	0.308	ns
FUNG Carb + Prop	-0.410	0.309	ns
FUNG Prop + Fenp	0.151	0.303	ns
TIME Post	-1.440	0.424	***
FUNG Carb . TIME Post	1.868	0.435	***
FUNG Prop . TIME Post	-0.601	0.422	ns
FUNG Carb + Prop . TIME Post	1.107	0.406	***
FUNG Prop + Fenp . TIME Post	-0.319	0.413	ns
SITE P'ferry 93 . TIME Post	1.827	0.527	***
SITE S'ford 94 . TIME Post	1.409	0.424	***
SITE N'ards 94 . TIME Post	1.962	0.431	***
SITE Mahee Isl 94 . TIME Post	1.962	0.431	***

S'ford = Strangford; P'ferry = Portaferry; N'ards = Newtownards

Mahee Isl. = Mahee Island

Carb = carbendazim (Derosal WDG); Prop = Propiconazole (Tilt 250 EC)

Carb + Prop = carbendazim + propiconazole (Hispor 45 WP)

Prop + Fenp = propiconazole + fenpropidin (Legend EC)

ns = not significant ($P > 0.05$); *** = very highly significant ($P < 0.001$)

Table 11. **Comparison between a PCR diagnostic method and bioassay for detection of benzimidazole resistance in *Rhynchosporium secalis* lesions.**

Test Method	Isolates tested	% benzimidazole resistant
Bioassay	75	3
PCR Diagnostic	87	9

Table 12. **Comparison between a PCR diagnostic method and bioassay for detection of benzimidazole resistance in *Rhynchosporium secalis* isolates.**

		Bioassay (ADAS Wolverhampton)		Total
		R	S	
PCR Diagnostic	R	16	3	19
(LARS)	S	14	126	140
Total		30	129	159

Septoria tritici: **Wheat leaf blotch**

Effect of reduced dose rate on selection for DMI (flutriafol) resistance

All fungicide treatments reduced the level of *S. tritici*. As expected the 1/4 dose rate of flutriafol was less persistent than the full rate (Table 13), and the flutriafol/chlorothalonil mixture gave the best overall control. A selection pressure clearly existed within populations at both sites, and in all three years.

Sensitivity of at least 650 isolates was assayed each year using micro-titre plate methodology. Considerable differences in the distribution of sensitivity to flutriafol occurred between plots at each site and it was important, therefore, to compare shifts within individual plots as part of the analysis to identify differences between treatments.

Nevertheless, populations in all plots contained about a 20-fold range in flutriafol sensitivity (0.06 - 1.2 $\mu g\ ml^{-1}$), although most of this variation was within a 2-3 fold range (Figure 6). Clearly, before flutriafol treatments were applied sufficient variation existed within populations for selection to act upon. This range of variation did differ between years; for example sensitivity distributions in *S. tritici* populations at Sonning in 1993 were all much narrower than in 1992 (compare Figure 6b with 6a). However, there was no evidence that populations established each year, from whatever inoculum source, were affected by use of fungicides at each site in previous years. Many isolates were characterised independently using a *S. tritici* specific DNA probe. All fingerprinted isolates were different, confirming that populations contained many different individuals, and not just a few clones.

Table 13a. **Sonning, 1992: Incidence of disease caused by *S. tritici* on leaf 2 of wheat cv. Riband at various dates after spraying on 14 May.**

Cultivar	Spray	Incidence (%) 23 May	2 June	9 June
Riband	0	6.1	30.3	72.1
	1/4	7.1	15.2	43.5
	1	3.3	6.9	22.0
	1+C	0	3.4	18.6

Table 13b. **Jealott's Hill, 1992: Incidence of disease caused by *S. tritici* on leaf 2 of cv. Riband at various dates after spraying on 6 May.**

Spray	Incidence (%) 17 May	23 May	2 June	9 June
0	14.3	74.6	94.2	100
1/4	5.7	42.6	89.0	98.9
1	1.4	27.3	72.2	92.5
1+C	*5.7	27.1	48.3	52.9

* high incidence due to uneven plant development

0 = water spray; 1/4 = 1/4 rate flutriafol;
1 = recommended dose of flutriafol; 1+C = flutriafol + chlorothalonil (Impact Excell)

No systematic shifts in flutriafol sensitivity were detected after spraying at any dose rate. Those changes in sensitivity that were observed (Figure 6) were usually towards greater sensitivity, and occurred in plots treated with water, as well as on treated plots. The only significant increase in resistance followed after treatment with a flutriafol/chlorothalonil mixture (Figure 6a; Sonning). In the final year (1994) the mixture treatment was replaced by split dose treatments of flutriafol (2 x 1/8th; 2 x 1/2) in order to prolong selection. No significant changes in sensitivity occurred in populations exposed to these split dose treatments.

In conjunction with a field trial in 1996 at LARS to evaluate the use of immunodiagnostics to determine spray threshold for Septoria control, the effect of different cyproconazole (Alto) dose rates on selection for DMI resistance was also explored. 105 isolates were assayed for cyproconazole, flusilazole and flutriafol sensitivity using the same microtitre plate method used to analyse *S. tritici* populations from the Sonning and Jealott's Hill sites. As with these earlier studies, differences in flutriafol sensitivity, but not cyproconazole or flusilazole sensitivities were detected in populations from different plots, but dose rate had no effect on selection for DMI resistance.

Monitoring and cross-resistance

Small surveys of flutriafol sensitivity were carried out in N. Ireland in 1992-3 and LARS in 1996. The LARS survey of 105 isolates confirmed the wide range in sensitivity (ED_{50} values 0.1 - 1.7 $\mu g\ ml^{-1}$) observed in populations from Sonning and Jealott's Hill. MIC values for isolates from N. Ireland ranged from 0.16 to > 1.25 $\mu g\ ml^{-1}$. Neither of these surveys detected any shift towards resistance when compared with the sensitivity distribution in populations surveyed in 1990 (Hollomon and Mapstone, unpublished results).

Flusilazole was more active *in vitro* against *S. tritici* than either cyproconazole or flutriafol. Comparison of Mean ED_{50} values for populations from different field plots showed a correlation between flutriafol and cyproconazole sensitivity, but not between flutriafol and flusilazole or between flusilazole and cyproconazole (Figure 7). Tests on a few selected isolates confirmed this lack of a link between flutriafol and flusilazole sensitivity.

Field performance of DMI fungicides against *Septoria tritici*

In addition to the trials at Sonning and Jealott's Hill, experiments carried out at other centres provided information on the performance of a wider range of DMI fungicides. Full-rate applications of cyproconazole, flusilazole or prochloraz all reduced foliage infection of *S. tritici* compared with an untreated control; well-timed applications of a quarter-rate gave similar levels of control initially, but were less persistent. However, products containing a DMI in a mixture, especially with chlorothalonil, were the most effective treatments.

Figure 6. Distribution of fungicide sensitivity (EC50) in each plot before and after spraying. The sample size (N) is shown in parentheses to the right of each plot; the plot is identified to the left; 0 - water spray; 1/4 - 1/4 rate spray with flutriafol; 1 - spray with full recommended dose of flutriafol; 1 + C - sprayed with a mixture of flutriafol and chlorothalonil. A scale appears in the middle of the figure.

| = Mean EC_{50} (mg ai l^{-1}). Each plot shows the extremes, quartiles and median of the distribution of the observed EC_{50}. *, ** : significant ($P \leq 0.05$ or $P \leq 0.01$) difference between before and after, according to a Kolmogorov-Smirnoff test.

a. 1992, cv. Riband

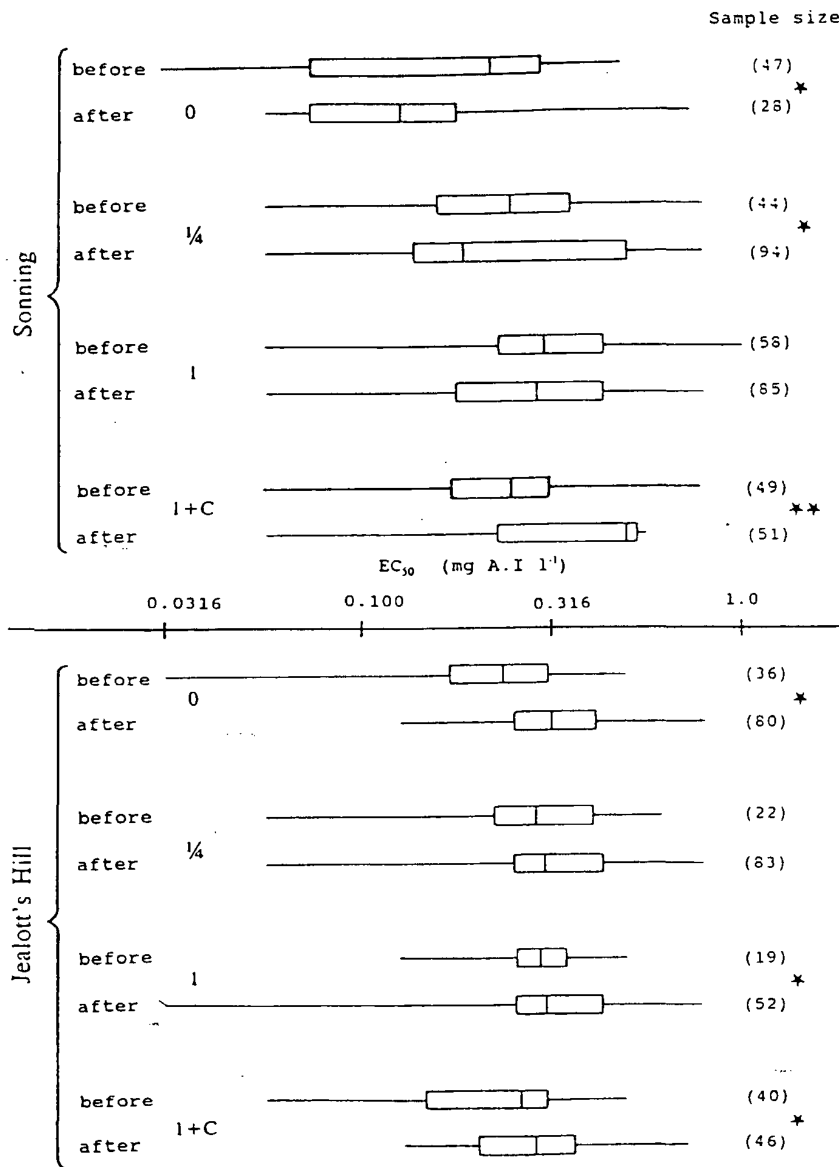


Figure 6b. **1993, cv. Riband**

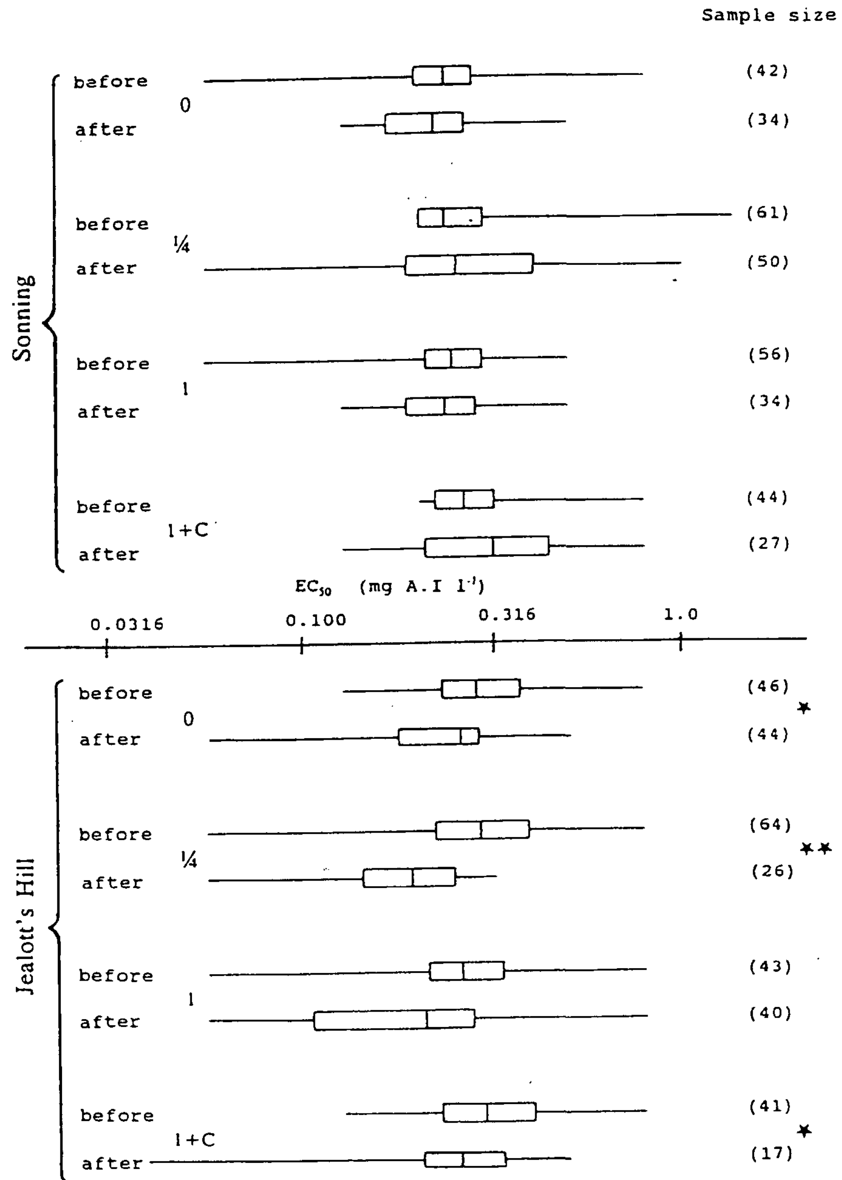
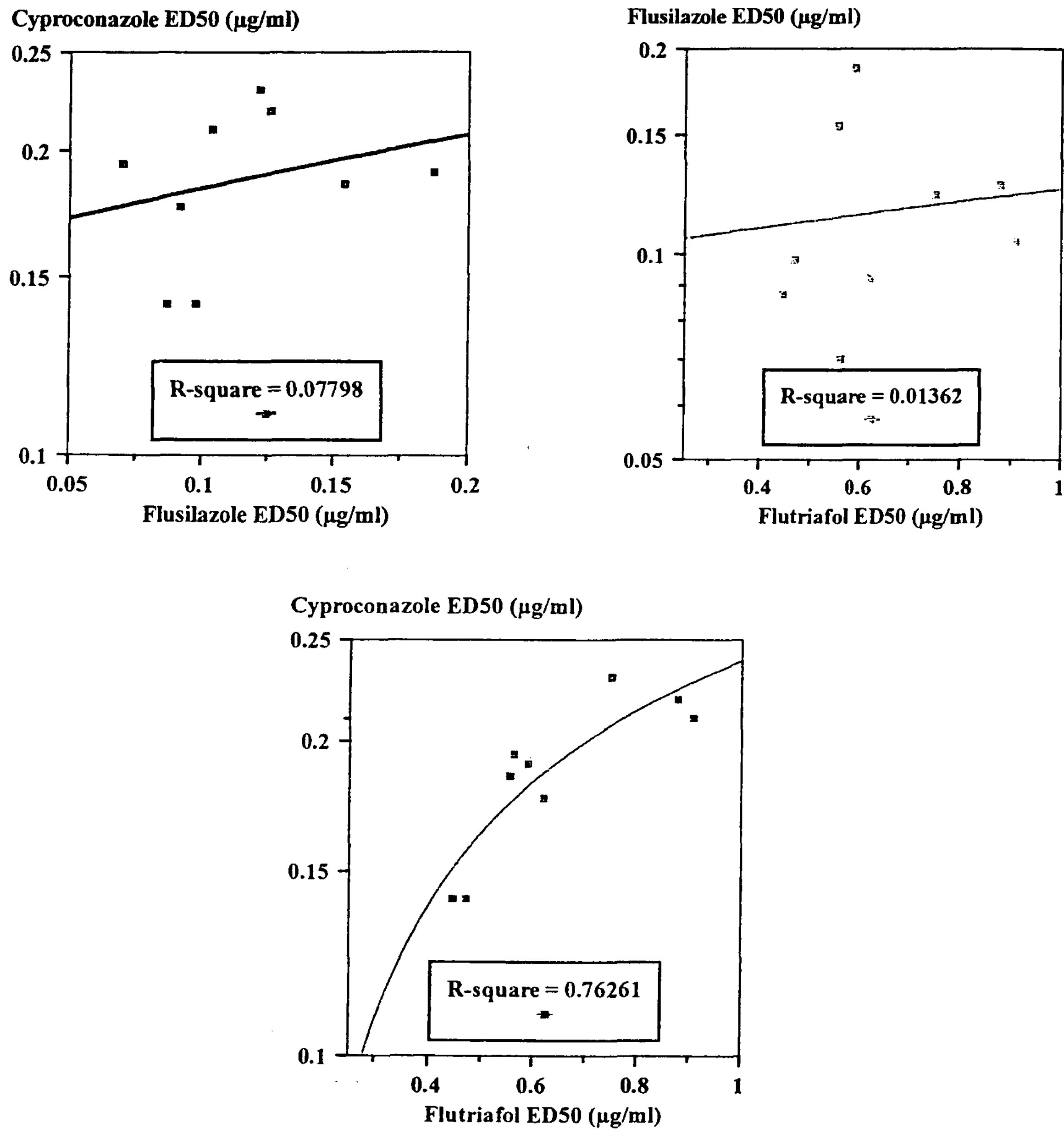


Figure 7. **Correlation between flutriafol, cyproconazole and flusilazole sensitivity in *Septoria tritici*. Each data point is the mean ED_{50} value for a population from a field plot. 105 isolates were assayed in total.**



Erysiphe graminis: cereal powdery mildew

Monitoring morpholine sensitivity

Fenpropimorph sensitivity of barley powdery mildew (*E. graminis* f.sp. *hordei*) has now been monitored since 1988. During this period significant differences in mean sensitivity have occurred between years, but the range in sensitivities has not changed, nor has there been any consistent shift towards lower sensitivity (Table 14). Tests of isolates supplied by Dr James Brown (John Innes Research Centre, Norwich; CC 1 $ED_{50} = < 0.015$ $\mu g\ ml^{-1}$; CC 139 $EC_{50} = 0.030$ $\mu g\ ml^{-1}$; CC 151 $EC_{50} = 0.318$ $\mu g\ ml^{-1}$) indicate that these isolates reflect the extremes of the normal wild-type distribution of fenpropimorph sensitivity. Between 1991 and 1993 there was also no change in tridemorph sensitivity. Monitoring fenpropidin sensitivity began in 1991 and although the sample size is not large, there does appear to be some decline in mean sensitivity, especially in 1994. Unfortunately no isolates were available for monitoring in 1995 because of the extremely hot summer, but results from a field experiment carried out in Scotland in 1995 (Table 16) suggests that fenpropimorph sensitivity may have declined somewhat also.

Table 14. Sensitivity of *Erysiphe graminis* f.sp. *hordei* (barley powdery mildew) to morpholine fungicides, 1988-94.

	Sensitivity (ED_{50} mg ml^{-1})					
	Fenpropimorph		Tridemorph		Fenpropidin	
Year	Mean	Range	Mean	Range	Mean	Range
1988	0.057	(0.007-0.119)		-		-
1989	0.021	(0.010-0.051)		-		-
1990	0.033	(0.008-0.115)		-		-
1991	0.082	(0.010-0.119)	0.116	(0.072-0.213)	0.051	(0.003-0.156)
1992	0.029	(0.010-0.108)	0.159	(0.009-0.246)	0.023	(0.007-0.030)
1993	0.076	(0.010-0.300)	0.180	(0.042-0.349)	0.090	(0.001-0.351)
1994	0.077	(0.024-0.195)		-	0.269	(0.195-0.382)

- No data

Some base line sensitivity data were also obtained for wheat powdery mildew between 1993-1995 (Table 15). Whereas fenpropidin is equally active against both cereal powdery mildews (compare Tables 14 and 15), fenpropimorph appears to be less effective against wheat than against barley powdery mildew. This supports experience from field crops where wheat mildew is more difficult to control with fenpropimorph than is barley mildew. As with fenpropidin sensitivity in barley powdery mildew there appears to be some decline in fenpropimorph sensitivity in 1995. However, the change was not large, and does not represent a significant change in mean sensitivity over the three years.

Table 15. **Sensitivity of *Erysiphe graminis* f.sp. *tritici* (wheat powdery mildew) to fenpropimorph and fenpropidin in 1993.**

Year	Sensitivity (ED_{50} mg ml^{-1})			
	Fenpropimorph		Fenpropidin	
	Mean	Range	Mean	Range
1993	0.167	(0.012-0.244)	0.003	(0.014-0.255)
1994	0.196	(0.062-0.324)	0.105	(0.017-0.403)
1995	0.327	(0.163-0.548)		-

- Not tested

Effect of dose rate on morpholine sensitivity

The effect of fenpropimorph at full rate, or reduced rates, on sensitivity was first explored in barley powdery mildew, and the results of the 1992 and 1993 field trials were presented by Zziwa and Burnett (1994). First sprays were applied when mildew was first seen in the crop; the second spray three weeks later. In 1995 these experiments were extended to include wheat mildew as well (Table 16). In both experiments sensitivity declined after treatment with fenpropimorph, but variability was large and shifts in sensitivity were not significant ($p = 0.05$). Any changes in sensitivity were not related to dose rate and after the second spray, incidence of wheat powdery mildew at least increased in the plots, but sensitivity returned to what it was before any fungicides were applied. Mixing fenpropimorph with propiconazole also had no effect on fenpropimorph sensitivity.

Table 16. **Effect of fenpropimorph dose rate on selection for resistance in cereal powdery mildew**

	Mean sensitivity (EC_{50} mg ml^{-1})				
Sampling time	Full dose*	Three quarters	Half	One quarter	SED
Barley mildew					
Before spraying	0.147	0.111	0.158	0.144	
After two sprays	0.174	0.207	0.328	0.191	± 0.0795
Wheat mildew					
Before spraying	0.263	0.131	0.209	0.193	
After one spray	0.348	0.263	0.274	0.328	
After two sprays	0.253	0.136	0.173	0.197	± 0.0934

* Full dose rate of fenpropimorph = 3.75 mg/ml

Puccinia striiformis: **yellow rust**

Monitoring morpholine sensitivity

268 isolates collected from UK wheat crops from 1961 onwards provided the data base for this survey; many had never been exposed to fungicides. Isolates varied widely in their sensitivity (Figure 8), although the frequency distribution was normal, and the mean sensitivity similar to that of the standard isolate used inall tests. There was no evidence that sensitivity had declined, even though fenpropimorph has been used in wheat crops since 1983 (Table 17). Sensitivity was unrelated to whether or not fenpropimorph had been used on crops from which isolates were obtained (Table 18). The ranking of isolates for sensitivity to fenpropidin was similar to their ranking for fenpropimorph (Table 19).

Figure 8. Baseline sensitivity distribution for fenpropimorph in *Puccinia striiformis*

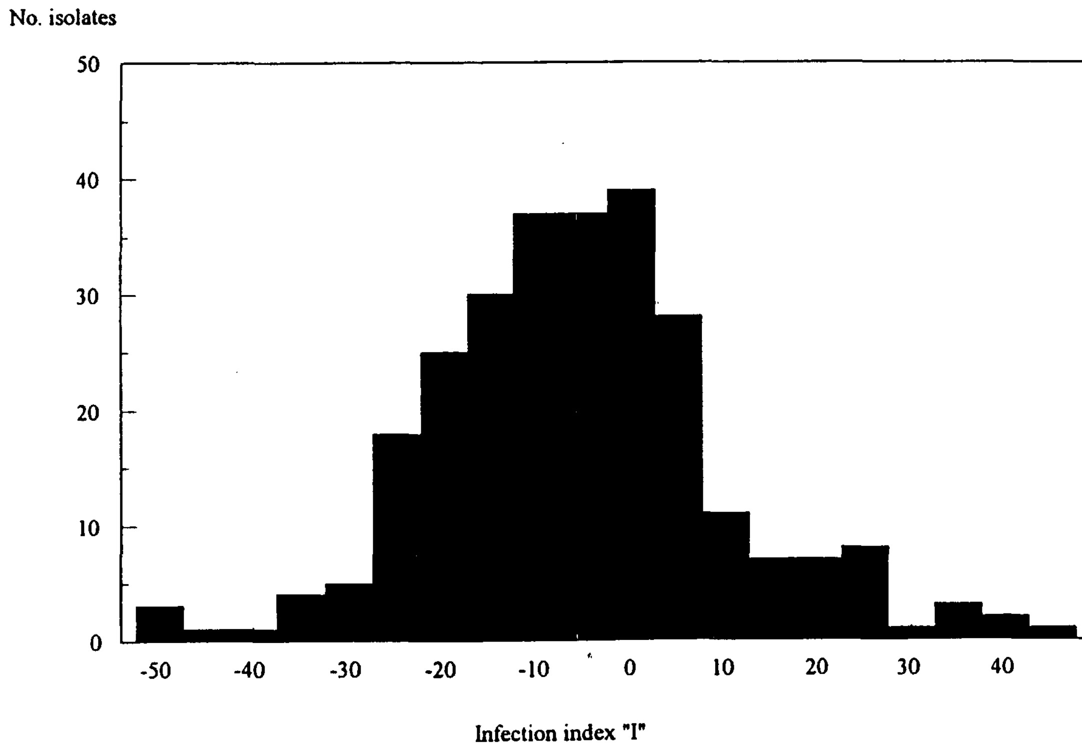


Table 17. Sensitivity of *Puccinia striiformis* to fenpropimorph: Changes with time

Year of isolation	No of isolates	Sensitivity (Mean Infection Index I)	Comparison	Significance
pre-1989	95	- 7.33	pre-'89 v '89	NS
1989	111	- 7.89	'89 v post '90	NS
post-1989	62	- 9.45	pre '89 v post '90	NS

Table 18. Effect of fungicide treatment on fenpropimorph sensitivity in *Puccinia striiformis*

Fungicide treatment	Number of isolates	Sensitivity (Mean Infection Index I)	Significance
None	230	- 8.33	NS
Fenpropimorph	38	- 6.82	

Table 19. Comparison of sensitivity to fenpropimorph and fenpropidin in *Puccinia striiformis*

Isolate	Sensitivity (Infection Index I)	
	Fenpropimorph (187.5 mg/ml)	Fenpropidin (375 mg/ml)
83/62	0	0
90/20	+ 14	+ 12
92/27	- 14	- 2
93/32	+ 7	+ 4

DISCUSSION

This report explores resistance questions surrounding fungicides used to control four of the most important UK cereal diseases, and where successful control is very dependent on continued good performance of fungicides. As expected, results emphasise that it is difficult to generalise about resistance, and each fungicide/disease combination must be treated separately. Above all resistance is a dynamic situation, which requires some form of constant monitoring to ensure that valuable fungicide resources are not wasted.

Prior to 1990, no benzimidazole (carbendazim) resistance was identified in *R. secalis* throughout the UK. Since then, carbendazim resistance has clearly become very widespread, and where selection is strong in the wetter regions of the country, performance suffers. Even so, the picture of carbendazim resistance is an erratic one in different parts of UK, and in some places good control is still achieved with carbendazim, especially if good timing is a feature of treatment. Resistance and fungicide use are not linked. Other factors must contribute to control difficulties however, and poor performance is often associated with areas where weather conditions favour leaf blotch and ensure maximum disease pressure, which in itself makes it difficult for fungicides to perform well. Unfortunately, mixing carbendazim with a DMI fungicide seems to have had no effect on slowing this selection for resistance.

Although these results were obtained using conventional bioassay methods, an experimental technique based on DNA probe technology provided some useful additional information. The general agreement between the two methods was very encouraging. The fact that direct detection of carbendazim resistance in lesions identified a higher frequency of resistance than detection in isolates from the same lesion growing *in vitro*, suggests that *R. secalis* may be heterokaryotic (Liu Bo *et al.*, 1994), and that resistance alleles are less stable than wild type ones. This might also account for the lower level of resistance detected in culture collections, and where DNA tests were carried out several generations after the corresponding bioassay. It is known, however, that more than one resistance allele may exist within *R. secalis* populations, and false negatives may be due to a corresponding resistance probe not being available. Whilst the potential advantages of DNA probe technology are clear in that they are rapid, can detect resistance in the presence of contaminating organisms, and identify particular resistance alleles, this report reveals the limitations of dot-blot methodology. High backgrounds, and the lack of quantification often made it difficult to identify positive hybridization events. For these reasons, a more robust micro-titre plate assay based on ELISA is being explored. Nevertheless, a major aim of resistance monitoring must be to increase the number of individual clones that can be evaluated, either microbiologically, biochemically or using molecular technologies, and this project has made a useful contribution towards achieving these aims. A higher throughput of isolates allows earlier detection of resistance which allows more scope for adapting fungicide use to combat further spread of the problem.

Although not specifically the subject of this research, it is clear that sensitivity of *R. secalis* to some DMIs has declined since the last report (Hollomon, 1992). In contrast to the erratic response of *R. secalis* to selection with benzimidazole fungicides, this shift in DMI sensitivity has occurred uniformly across the country, and seems unrelated to DMI use. It also contrasts with the response of *S. tritici* to selection of field populations with these two fungicide groups. Benzimidazole resistance in wheat blotch populations is widespread, and occurs at high frequency, whereas selection of DMI resistance has been limited and, as yet, has not caused any performance difficulties. Nevertheless, cross-resistance patterns to different DMI fungicides are very similar in these two pathogens. Low resistance factors are always

associated with prochloraz, tebuconazole, and flusilazole reflecting perhaps, the relative strength of different resistance mechanisms towards various DMI fungicides. In practice, mixtures of DMIs with morpholine fungicides have given good control of *R. secalis*, and it is suggested that this mixture strategy is one option for countering any further selection for DMI resistance.

Despite continuous use of morpholine fungicides to control cereal powdery mildews for over 25 years, there is still no evidence that selection for resistance occurs, at least to a level that adversely affects performance (Hollomon, 1994). As illustrated in this report, the mean sensitivity of a mildew population may differ between years, and possibly between sites, but any shifts are small, and overall resistance factors were never more than 10-fold. This compares with resistance factors of 100-fold, or more, to benzimidazole, phenylamide, azole and aminopyrimidine fungicides, in pathogen populations where resistance has caused performance difficulties. Reports of morpholine resistance (Brown *et al.*, 1991) in cereal mildews do not reflect performance problems, and appear to refer to analysis of mildew isolates from extremes of the wild-type sensitivity distribution. Isolates of wheat powdery mildew with reduced sensitivity to morpholines (fenpropimorph) had lower pathogenic fitness than normal wild-type ones, and this may account for the failure to select high levels of resistance (Engels and DeWaard, 1994).

Repeated fungicide treatments, but at reduced doses, is a common strategy to manage disease control in cereals. A major concern amongst growers and advisers involved in crop protection, is the effect these repeated low doses may have on selection for resistance. Theoretical evidence from mathematical modelling of the predicted response of pathogen populations to different selection intensities is conflicting, with some authors concluding, at least with agricultural relevant dose rates, any harmful effect of reduced dose rates on selection for resistance was negligible (Shaw, 1989). Others (Milgroom and Fry, 1988) concluded the opposite, and that dose rate was important.

Evidence from field experiments is no less conflicting, but some of it suggests that split doses of morpholine fungicides do select somewhat lower levels of sensitivity in cereal mildews, albeit no more than a 2-3-fold decline (Engels and DeWaard, 1994; Forster *et al.*, 1994; Schulz, 1994). These studies were all conducted with powdery mildews, and in no case was performance reduced. Apart from the difficulty of assaying many mildew isolates, the effect of wind-borne migration of conidia is a problem in evaluating selection induced changes, especially when the same fungicides are being used commercially on neighbouring farms. Nevertheless, difficulties in generalising on fungicide resistance issues between different pathogen/fungicide combinations, make it essential that data are collected from mildew trials designed to explore the effect of reduced dose rates on resistance. (In none of the experiments reported here did dose rate affect the sensitivity to morpholine fungicides in cereal powdery mildews.) However, a decline in sensitivity was sometimes detected following repeated applications of fenpropimorph for mildew control but changes were small. Additional sprays even increased sensitivity in some cases.

S. tritici offers many advantages over mildew in studies of dose rate. Not only is it a less mobile pathogen once ascospore infection has taken place in the autumn, but significant variation in DMI sensitivity is available for selection to operate on. The pathogen can also be grown in culture allowing testing of large numbers of isolates. Nevertheless, there was no evidence of selection for resistance with reduced doses. Indeed there was no evidence of selection at all, despite significant levels of control which presumably preferentially removed

the least sensitive isolates from the population. One reason for this may relate to very mobile azoles, such as flutriafol, used in this field experiment. These readily accumulated in leaf margins and tips giving good disease control in these regions, but leaving the remainder of the leaf unprotected. Consequently, there would be a reservoir of a selected individual which would figure prominently in isolation and testing procedures.

As in the earlier report dealing with DMI sensitivity in brown rust (Hollomon, 1992), there was no evidence that yellow rust populations were any less sensitive to morpholines in the 1990s, than they were before their use on wheat. The reasons for this stability is not clear, but it may be related to the dikaryotic status of rust fungi, compared with haploid ascomycete pathogens where selection for resistance is quite common.

CONCLUSIONS

1. Benzimidazole resistance is now widespread in *R. secalis* populations, and can cause performance difficulties, especially in areas where weather conditions favour the disease.

2. Further selection for resistance to some DMIs has occurred, and growers should mix these with a morpholine fungicide. Newer fungicides with different modes of action are becoming available, with good activity against *R. secalis*, but as yet no independent studies of their use in DMI mixtures for *R. secalis* control have been reported.

3. Although fenpropimorph and fenpropidin sensitivity may recently have declined in both wheat and barley powdery mildew, changes have been small, and are unlikely to affect performance.

4. Reduced dose rates, at least within agriculturally relevant dose levels, did not select for resistance on cereal powdery mildews, and *S. tritici*.

5. A wide range of sensitivities to DMI fungicides exists in *S. tritici* populations, but these are stable and performance of these fungicides remains good.

6. Yellow rust isolates varied widely in their sensitivity to morpholines but sensitivity has not declined over a thirty year period, and control remains good.

FUTURE DIRECTIONS FOR RESEARCH

So far, resistance has caused few practical problems in cereal disease control. In part, this is due to the success of the research funded by Government, the Agrochemical Industry, and HGCA, which has identified potential problems early, and provided the framework of understanding needed to effectively manage resistance. Investment by the Agrochemical Industry in developing fungicides with different modes of action has also been important, and has allowed use of fungicide mixtures involving partners with different modes of action to be the major anti-resistance strategy. Since cross-resistance patterns follow mode of action this has been a convenient and simple way to explain to users the basis of the strategy, although in reality it is the use in mixtures of fungicides which select different mechanisms of resistance that is important. The realisation that each DMI fungicide may select a different mechanism of

resistance opens the way to use DMI mixtures to improve overall disease control without causing resistance. **This strategy needs to be evaluated in further field experimentation.**

The next few years could well see a major change in the way fungicides are used. Many new fungicides are either available now, or will be by 1997, and many others are in various stages of development. Several combine systemic movement with protectant and various degrees of eradicant activity. They are persistent and provide control for long periods. **Independent research is needed to explore how best to use these new fungicides most effectively in strategies with older DMI fungicides to provide broad spectrum, "top up" disease control in the later stages of crop development. Resistance management must be a component of this work.** It will be important for growers to use these new fungicides in ways which preserve the activity of older, but cheaper, fungicides.

Dose rate is still a major issue in resistance management. **Extending the work described in this report to include less mobile azole fungicides would be useful in helping to operate sustainable, and cost-effective disease control strategies.**

Registration requirements now need baseline sensitivity data to be provided for registration, and there is no need for HGCA to duplicate this in its research funding. Registration also requires an outline of anti-resistance strategies, **and it would seem that there is a case for independent evaluation of these strategies.**

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Many other publications have been produced for trade journals, of various sorts. The work has also provided a major input into a leaflet on Fungicide Resistance published by the UK Fungicide Resistance Action Group in 1996.

This project has supported the successful training of two post-graduate students.

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