PROJECT REPORT No. 290

FUNGICIDE PROGRAMMES FOR EFFECTIVE CONTROL OF RHYNCHOSPORIUM ON WINTER BARLEY

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by

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CONTENTS

ABSTRACT

The control of Rhynchosporium (leaf blotch), currently the most economically-important disease of barley, is heavily dependent on fungicides. The DMI fungicides were the key element of control programmes, but the effectiveness of older DMIs has been impaired by selection of resistant strains. During the 1990s, MBC-resistant strains of *Rhynchosporium secalis* developed and these are now too widespread for MBCs to be suitable partners for DMIs in anti-resistance strategies.

To prolong the effective life of the newer, more active DMIs, two-spray fungicide programmes were evaluated for their effectiveness in combining good control of Rhynchosporium with prevention of the build-up of DMI-resistant pathogen strains. Programmes were based on the DMI epoxiconazole ('Opus') alone or in combination or alternation with three partner fungicides with different modes of action, fenpropimorph ('Corbel', a morpholine), cyprodinil ('Unix', an anilinopyrimidine), azoxystrobin (a strobilurin, 'Amistar').

In a series of field trials in Northern Ireland and South-west England over the three years 1998-2000 performance of programmes was assessed in terms of disease control, prolongation of green leaf area and yield and effects on DMI sensitivity were monitored by testing isolates of the pathogen collected before and after fungicide application.

Across the six trials, all fungicide treatments reduced disease significantly compared with the untreated control. Epoxiconazole used alone gave the poorest disease control and lowest green leaf area, but did increase yield. The three partner fungicides all improved disease control and yield. Programmes based on two applications of half-rate epoxiconazole with half-rate azoxystrobin or cyprodinil performed best overall, cyprodinil being marginally more effective in terms of disease control, while azoxystrobin combinations achieved the best yields.

In the majority of trials, DMI sensitivity of *R. secalis* isolates was lower after fungicide treatment than before and the least sensitive isolates came from the plots treated with two half-rate applications of epoxiconazole alone. There was no consistent difference in effects on epoxiconazole sensitivity between the three partner fungicides, but all tended to reduce selection for resistance compared with two half-rate applications of epoxiconazole alone.

It is concluded that selection for DMI resistance is continuing to occur in *R. secalis*, but that use of a partner fungicide helps to slow down the process, while not preventing it. With the range of pathogen sensitivities found in the present study, a DMI such as epoxiconazole is a useful component of a control programme for *R. secalis*, but must be supplemented by a partner fungicide. Fungicides with different modes of action from the DMIs from the strobilurin (QoI) and anilinopyrimidine group are the most effective partners in terms of disease control and yield.

SUMMARY

Control of Rhynchosporium on barley has relied on the use of DMI (**D**e**M**ethylation **I**nhibitor) fungicides since their introduction in the 1970s. Varietal resistance can complement the use of fungicides, but is insufficient on its own, since although some barley varieties are resistant to specific Rhynchosporium races, none currently grown in the UK is resistant to all races. The sensitivity of many plant pathogens to DMI fungicides tends to reduce over time as more resistant strains are selected and this process gradually impairs the effectiveness of DMIs in controlling them. In the case of *Rhynchosporium secalis*, previous HGCA-funded projects demonstrated that this occurred during the 1980s and early 1990s, leading to older DMIs becoming virtually ineffective. Newer DMIs such as epoxiconazole are inherently more active against the pathogen, but it seemed likely that in time their performance too would be impaired.

Anti-resistance strategies are designed to slow down the selection of resistant pathogen strains so that disease control is sustained and the costs of lost production and use of replacement products are not sustained. The corner-stone of such strategies is the use of programmes based on fungicides differing in their modes of action or mechanisms of resistance. In the case of Rhynchosporium, until the 1990s, the most appropriate partners were fungicides from the morpholine or MBC groups. MBCs had good activity against *R. secalis*, while morpholines, although not particularly active against this pathogen when used alone, had proved to have a synergistic effect when used in combinations with DMIs. During the early 1990s, MBC-resistant strains of *R. secalis* were detected for the first time and rapidly became widespread, particularly in the wetter northern and western parts of the UK such as Northern Ireland. Such strains are now too common for MBCs to be suitable partners for DMIs in anti-resistance strategies.

The introduction in the mid-1990s of the strobilurins and anilinopyrimidines provided fungicides with novel modes of action and activity against *R. secalis*, which could be used as components of anti-resistance programmes. Such programmes would potentially not only provide sustainable disease control and help to reduce selection for resistance to the DMIs, but also reduce the risk of development of resistance to the newer types of fungicide.

The objective of this project was to determine the most effective programmes to combine good control of Rhynchosporium with prevention of the build-up of DMI-resistant pathogen strains in order to prolong the usefulness of the newer, more active DMI fungicides. Two-spray fungicide programmes were devised based the DMI epoxiconazole (‘Opus’) alone or in combination or alternation with three partner fungicides with different modes of action:

- fenpropimorph (‘Corbel’) - a morpholine
- cyprodinil (‘Unix’) - an anilinopyrimidine
- azoxystrobin (‘Amistar’) - a strobilurin.

These fungicides were selected at the start of the project on the basis of their availability as single active ingredient products and activity against *R. secalis*. Programmes based on them were evaluated in a series of six field trials in Northern Ireland and South-west England over the three years 1998-2000. The same set of 12 treatments was used for all trials to allow analysis of overall results across years and sites. At both T1 and T2, fungicides were either applied singly or in combination. Where they were applied in combination the rate was half that recommended by the manufacturer. The trials were designed so that the total amount of epoxiconazole per set of treatments was always 125 g.

Performance was assessed in terms of disease control, prolongation of green leaf area and, in the Northern Ireland trials, yield. Effects on DMI sensitivity were monitored by collecting samples of infected leaves from plots before and after treatment, isolating the pathogen and testing its sensitivity to epoxiconazole *in vitro* using ELISA-plate or poisoned agar plate assays.

In the overall analysis across the six trials, all fungicide treatments reduced disease significantly compared with the untreated control. Epoxiconazole used alone (as a single full-rate application at T1 or as two half-rate applications at T1 and T2) gave the poorest disease control and lowest green leaf area and in some trials these were not significantly different from the untreated control. However, epoxiconazole alone did increase yield. The three partner fungicides all improved disease control and yield. There was no difference between programmes where full-rate epoxiconazole at T1 was followed by full-rate partner at T2 and those where half-rate epoxiconazole + half-rate partner were applied at both T1 and T2. Programmes where full-rate epoxiconazole at T1 was followed by half-rate epoxiconazole + half-rate partner at T2 were less effective, showing the effect of the amount of partner fungicide applied. The programmes based on two applications of half-rate epoxiconazole with half-rate azoxystrobin or cyprodinil performed best overall, cyprodinil being marginally more effective in terms of disease control, while azoxystrobin combinations achieved the best yields.

Sufficient isolates for DMI sensitivity evaluation were obtained from four trials (Northern Ireland and England trials in both 1998 and 2000). In three out of the four trials, sensitivity was lower in *R. secalis* isolates collected after fungicide treatments were applied than in those collected before treatment demonstrating that selection for reduced sensitivity can occur within a single season. Also in three out of four trials, the least sensitive isolates came from the plots which had received two half-rate applications of epoxiconazole. In contrast, there was no evidence that a single full-rate epoxiconazole application had a greater tendency to select for resistant fungicide strains than the other fungicide programmes. There was no consistent difference in effect on epoxiconazole sensitivity between the three partner fungicides, but all tended to reduce selection for resistance compared with the application of two half-rate applications of epoxiconazole alone.

It is concluded that:

- selection for DMI resistance is continuing to occur in *R. secalis.*
- with the range of pathogen sensitivities found in the present study, a DMI such as epoxiconazole is a useful component of a control programme for *R. secalis*, but must be supplemented by a partner fungicide.
- fungicides with different modes of action from the DMIs from the strobilurin (QoI) and anilinopyrimidine group are the most effective partners in terms of disease control and yield.
- use of a partner fungicide helps to reduce selection for DMI resistance, but cannot prevent it.

While DMI use continues, DMI sensitivity will continue to decline, although the effective life of these fungicides can be prolonged by avoiding their use as sole active ingredients and particularly by avoiding repeated use of half-rates of DMIs alone. In some regions, DMIs may no longer be worthwhile components of *R. secalis* control programmes, but it is imperative that sensitivity is assessed before assuming that their use is not justified, since reliance on strobilurin or anilinopyrimidine fungicides alone in itself poses potential risks of resistance.

INTRODUCTION

Rhynchosporium disease of barley (also known as leaf blotch or scald) is an increasing problem in the UK. During the 1970s and 1980s, it was effectively controlled by fungicides from two groups, the benzimidazoles and the DMIs. Shortly after the benzimidazoles were introduced in the 1960s, highly benzimidazole-resistant strains of some fungal pathogens were selected, but for over 20 years this did not occur in *R. secalis*. In the late 1970s, the introduction of the first DMI (**D**e**M**ethylation **I**nhibitor) fungicides, often referred to as 'azoles' or 'triazoles', were a major advance in cereal disease management, providing broad spectrum control of all major fungal pathogens. Since then a very extensive range of DMIs has been developed, which differ in the spectrum of diseases controlled, the degree of systemicity and their inherent activity, the most recently-introduced being effective at much lower doses than their predecessors.

Resistance to DMI fungicides impaired their performance in controlling barley and wheat powdery mildews during the 1980s (e.g. Wolfe, 1984; Heaney *et al.*, 1986; Clark, 1992) and led to the adoption of strategies in which DMIs were used in mixtures with fungicides with different modes of action. HGCA-funded work showed that the sensitivity to DMIs of the UK *R. secalis* population had also declined in the period 1986-1990 and that this was paralleled by reduced disease control (Kendall *et al*., 1993). For control of *R. secalis*, combinations of DMIs and benzimidazoles appeared the most effective control option and also provided an anti-resistance strategy. Unfortunately, at the beginning of the 1990s, HGCA-funded work in England and Northern Ireland detected benzimidazole resistance in *R. secalis* for the first time and it rapidly became both common and widespread (Taggart *et al*, 1998; Taggart *et al*., 1999). Thus continued reliance on either DMIs alone or DMI + carbendazim mixtures was concluded to pose risks in terms of selection for resistance and reduced control.

The use of DMI + morpholine mixtures for *R. secalis* control provided one possible alternative, because even though morpholines alone are relatively ineffective against this pathogen, they have a synergistic effect in controlling *R. secalis* when used in combination with DMIs. However, the introduction in the mid-1990s of the strobilurins and anilinopyrimidines provided fungicides with novel modes of action and activity against *R. secalis*, which could be used as components of control programmes.

At the time this project started in early 1998, the possibility that strains of pathogens resistant to these two fungicide groups would develop was recognised. In the case of the strobilurins, laboratory generation of a resistant strain of *Septoria tritici* had been demonstrated, but although ten times less sensitive to azoxystrobin than the wild-type *in vitro*, it was controlled better than the wild-type *in vivo* (Ziogas *et al.*, 1997). With the anilinopyrimidine group, highly resistant strains of *Botrytis cinerea* had already been obtained from the field in France and Switzerland (Leroux & Gredt, 1995; Forster & Staub, 1996; Hilber & Schuepp, 1996). Cross resistance was shown to occur between the three available anilinopyrimidines

(cyprodinil, mepanipyrim and pyrimethanil) and in some cases decreased sensitivity was associated with reduced disease control.

Shortly after the project began, during summer 1998, resistance to the strobilurin fungicides developed in wheat powdery mildew in northern Germany and was subsequently detected in the UK (Napier *et al.*, 2000). Since then strobilurin resistant strains have been identified in several other plant pathogens including barley powdery mildew; in almost all cases resistance has been associated with a single major gene change (Heaney *et al.* 2000; Gisi *et al.*, 2002). To date there have been no published reports of strobilurin resistance in other cereal pathogens (FRAC, 2001). With respect to the anilinopyrimidine fungicides, isolates of the cereal eyespot pathogens *Tapesia yallundae* and *T. acuformis*, with reduced sensitivity to cyprodinil have been isolated from UK trial sites monitored over several years and although the reduction in sensitivity was not clearly associated with a decline in disease control it was concluded that there is clearly a resistance risk in eyespot to cyprodinil (Babij *et al.* 2000).

This project was designed to explore the use of morpholine, strobilurin and anilinopyrimidine as mixture partners for DMIs with the dual objectives of managing resistance to DMI fungicides and achieving sustainable disease control. In addition, the use of DMIs with strobilurins and anilinopyrimidines should help to reduce the risk of *R. secalis* strains resistant to the latter fungicides.

OBJECTIVES

To determine the most effective mixtures, based on combinations of new fungicides, DMIs, and morpholines to combine good control of Rhynchosporium with prevention of the build-up of resistant pathogen strains in order to prolong the usefulness of the DMI fungicides.

MATERIALS AND METHODS

Location of field trial sites

Six field trials were carried out in commercially-grown crops of winter barley. In each of the years 1998-2000, there was one trial in South-west England and one in Northern Ireland. In 1998, the trials were near West Bagborough, Somerset (cv. Epic) and Temple, Co. Down (cv. Fighter). In 1999, they were near Exeter, Devon (cv. Fighter) and Gransha, Co. Down (cv. Pastoral). In 2000, near Wembury, Devon (cv. Fighter) and Killinchy, Co. Down (cv. Pastoral).

Layouts and treatments

The trials were laid out as fully randomised blocks (3 blocks x 12 treatments). Plot sizes varied slightly according to the land available, but were generally *c*. 5 x 10 m.

Combinations of a DMI fungicide, epoxiconazole, a morpholine, fenpropimorph, a strobilurin, azoxystrobin, and an anilinopyrimidine, cyprodinil, were used. These fungicides were selected on the basis of their good activity against *R. secalis* (either alone or in combinations) and availability as formulations containing single active ingredients (Table 1). Rates of fungicides were chosen such that all treated plots received the same total amount of epoxiconazole (125 g) either as a single or a split application. A common set of 12 treatments (Table 2) was imposed at all sites. Fungicide treatments were applied twice (T1 and T2), as far as possible at growth stages (GS) 31-33 and GS 51-59 (Table 3) in *c.* 250 litres/ha using Oxford Precision Sprayers.

Disease and green leaf area assessments

Rhynchosporium was the main disease at all sites. Ten randomly sampled tillers were collected from each plot on at least two occasions from each trial (Table 4), just before or immediately after the second fungicide application (GS 50-61) and again approximately 3 weeks later (GS 63-75). The percentage area affected by leaf blotch was estimated on the top three leaves of each tiller using the key described by James (1971) and the mean of the ten samples of each leaf was recorded. Similarly, the percentage green area was estimated for 10 tillers per plot and the mean green leaf area calculated.

Grain yield and quality assessments

Yield data were collected from the three Northern Ireland field trials and the 1998 trial in South-west England. A central 2 m swath was harvested from each plot and grain yield (at 15% MC) determined. In Northern Ireland, sub-samples were oven dried (80°C) and used to estimate thousand grain weight and specific weight (both at 0% MC).

Table 1. Fungicide formulations used in the trials

Proprietary name	Active ingredient	Rate of a.i.	Formulation type	Manufacturer
'Amistar;	azoxystrobin	250 g/l	SC	Syngenta
'Corbel'	fenpropimorph	750 g/l	EC	BASF
'Opus'	epoxiconazole	125 g/l	SC	BASF
'Unix'	cyprodinil	750 g/l	WG	Syngenta

Table 2. Fungicide treatments at two timings

Treatment number	Active ingredient (rate g/ha)			
	T1 treatment		T2 treatment	
1	none		none	
2	epoxiconazole (125)		none	
3	epoxiconazole (62.5)		epoxiconazole (62.5)	
4	epoxiconazole (125)		fenpropimorph (750)	
5	epoxiconazole (62.5)	+ fenpropimorph (375)	epoxiconazole (62.5)	+ fenpropimorph (375)
6	epoxiconazole (62.5)		epoxiconazole (62.5)	+ fenpropimorph (375)
7	epoxiconazole (125)		azoxystrobin (250)	
8	epoxiconazole (62.5)	+ azoxystrobin (125)	epoxiconazole (62.5)	+ azoxystrobin (125)
9	epoxiconazole (62.5)		epoxiconazole (62.5)	+ azoxystrobin (125)
10	epoxiconazole (125)		cyprodinil (510)	
11	epoxiconazole (62.5)	+ cyprodinil (255)	epoxiconazole (62.5)	+ cyprodinil (255)
12	epoxiconazole (62.5)		epoxiconazole (62.5)	+ cyprodinil (255)

Table 3. Details of fungicide application dates

Year	Location	Treatment 1		Treatment 2	
		Growth stage	Date	Growth stage	Date
1998	N Ireland	GS 31	20 April	GS 51	19 May
	England	GS 31/32	17 April	GS 53	13 May
1999	N Ireland	GS 32	30 April	GS 55	20 May
	England	GS 32	19 April	GS 69/71	18 May
2000	N Ireland	GS 32	28 April	GS 57	17 May
	England	GS 33/37	27 April	GS 59/61	25 May

Table 4. Details of timing of disease assessment

Year	Location	Assessment post 2nd fungicide application		Harvest date
		Growth stage	Date	
1998	N Ireland	GS 71	15 June	15 August
	England	GS 71	2 June	7 August
1999	N Ireland	GS 63	8 June	28 July
	England	GS 75	7 June	26 July
2000	N Ireland	GS 69	6 June	29 August
	England	GS 75	13 June	August

Sensitivity to epoxiconazole

Before fungicide treatments were applied, leaves with lesions of *R. secalis* were collected randomly from throughout the trial area to allow the initial sensitivity of the *R. secalis* population at each site to be determined. Approx. 20-30 days after the second fungicide application, 50-100 lesion-bearing leaves were collected from each plot. The leaves were air-dried at room temperature in paper bags and stored either at room temperature or frozen. These were used to establish up to 10 isolates of *R. secalis* from each plot.

R. secalis was isolated from lesions on surface-sterilised leaf sections placed on antibiotic yeast malt agar (YMA; yeast extract 10 g, malt extract 10 g, Oxoid Agar No.3 6 g, chloramphenicol 100 mg, streptomycin sulphate 200 mg, iprodione 10 mg, distilled water 1 litre) in Petri dishes and incubated under white light at 18°C. Leaf sections were examined periodically after from 3 to 21 days' incubation. *R. secalis* conidia or mycelia were picked from the leaf surfaces using a sterile needle and transferred to antibiotic Czapek Dox agar plates with mycological peptone (CDA; Czapek Dox agar 46 g, mycological peptone 5 g, chloramphenicol 100 mg, streptomycin sulphate 200 mg, distilled water 1 litre).

To provide the inoculum for the sensitivity assay, spores from CDA plates of each isolate were transferred into 10 ml of glucose/yeast broth (yeast extract 2 g, glucose, 10 g, chloramphenicol 100 mg, distilled water 1 litre) and incubated overnight at 4°C. ELISA plates containing a series of concentrations of epoxiconazole were prepared by pipetting aliquots (10 µl) of solutions of technical grade epoxiconazole in methanol into the wells and allowing the solvent to evaporate. Inoculum (100 µl) was added to each well, giving final epoxiconazole concentrations of 0, 0.041, 0.123, 0.37, 1.11, 3.33, 10 and 30 mg a.i./litre. Each isolate was tested in duplicate wells for each epoxiconazole concentration. ELISA plates were sealed and incubated for 14 d at 16°C in darkness and then growth assessed by measuring the absorbance at 450 nm in an ELISA plate reader. Sensitivity to epoxiconazole was expressed in terms of the minimum inhibitory concentration (MIC), the lowest epoxiconazole concentration which inhibited growth of *R. secalis* or as ED_{50} values calculated from the dose response.

In 2000, in England, an alternative sensitivity assay was used in which, isolates were tested on agar plates containing a series of concentrations of epoxiconazole (0, 0.041, 0.123, 0.37, 1.11, 3.33, 10 and 30 mg a.i./litre), using three replicate plates per isolate per concentration. After incubation for 10 d at 18°C, growth was assessed and MIC values estimated.

Analyses

Data from disease and yield assessments were subjected to analyses of variance.

RESULTS

Effect of fungicide treatments on disease development and green leaf area

The disease assessments made approx. 3 weeks after the second fungicide application (in early June) between GS 63 and GS 75, depending on the season (Table 4) proved most appropriate for discriminating between treatments. The results of these assessments are shown in Tables 5 and 6 and depicted in Figures 1 and 2. The amounts of infection varied greatly between seasons and sites, being low in Northern Ireland in 1999 and particularly in 2000. The untreated control did not invariably have the highest levels of *R. secalis* infection on leaves 1 and 2; in some assessments more infection occurred on the epoxiconazole alone treatments (either Tr. 2 full-rate epoxiconazole at T1 only or Tr. 3 half-rate epoxiconazole at T1 and T2).

In order to evaluate which programmes were the most effective overall, all six trials over the three years were analysed together, the trial series with its common treatments having been designed to permit this. The overall analysis revealed a very highly significant ($P<0.001$) effect of treatment on Rhynchosporium infection (Fig.3). All of the treatment combinations gave a significant reduction in disease compared with the untreated control. The treatments containing epoxiconazole alone (Tr. 2, Tr. 3) and the epoxiconazole/epoxiconazole + fenpropimorph treatment (Tr. 6) were the least effective.

The extent of disease development in the other treatments was related to the amount of the partner fungicide applied, but not to the timing of application of the components; there was no difference in effectiveness between applying half-rate epoxiconazole + half-rate partner at both T1 and T2 compared with applying full-rate epoxiconazole at T1 followed by full-rate partner fungicide at T2. In every case, greater disease development occurred when half-rate epoxiconazole was applied at T1 followed by half-rate epoxiconazole + half-rate partner at T2.

There was no significant difference in terms of Rhynchosporium development between plots treated with the combinations of the three partner fungicides except where the partner was applied only at half-rate with epoxiconazole at T2, when fenpropimorph was less effective than cyprodinil and azoxystrobin.

The green leaf area assessments for leaf 2 (leaf 3 for the 1998 Northern Ireland trial) are shown in Table 7. Analysis of all six trials over the three years revealed a very highly significant ($P<0.001$) effect of treatment and a broadly similar pattern to the disease assessments in terms of performance (Fig. 4). The epoxiconazole alone treatments had the lowest green leaf areas apart from the untreated control from which they did not differ significantly ($P<0.05$). Tr. 8 (half-rate azoxystrobin + epoxiconazole at T1 and T2) and the epoxiconazole and cyprodinil combinations (Tr. 10 and Tr. 11) achieved the largest green leaf areas.

Table 5. Effect of treatments on *R. secalis* infection on leaves 1 and 2, Northern Ireland trials

Tr. no.	Treatment	*Rhynchosporium secalis* infection (%)					
		1998 (GS 71)		1999 (GS 63)		2000 (GS 69)	
		Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2
1	Untreated	9.7	34.1	5.5	9.2	0.1	2.1
2	E/none	3.9	24.1	1.8	2.7	0.0	0.8
3	E/E	2.5	22.1	5.6	10.7	0.3	1.2
4	E/F	0.9	11.8	1.9	3.8	0.0	1.1
5	E+F/E+F	0.6	9.5	1.3	2.8	0.0	2.1
6	E/E+F	0.9	30.1	0.2	0.8	0.0	1.7
7	E/A	1.2	15.9	1.0	1.5	0.0	0.0
8	E+A/E+A	1.2	12.2	0.2	2.3	0.0	1.0
9	E/E+A	2.9	19.1	0.7	2.1	0.0	1.2
10	E/C	0.5	9.1	0.6	0.2	0.0	1.1
11	E+C/E+C	0.9	6.9	0.8	2.5	0.0	0.5
12	E/E+C	1.0	8.2	1.5	3.8	0.0	1.4
L.S.D. (P<0.05)		3.13	n/a	n/a	5.60	n/a	n/a

n/a not applicable: L.S.D. values were only calculated where the effect of treatment was significant (P<0.05).

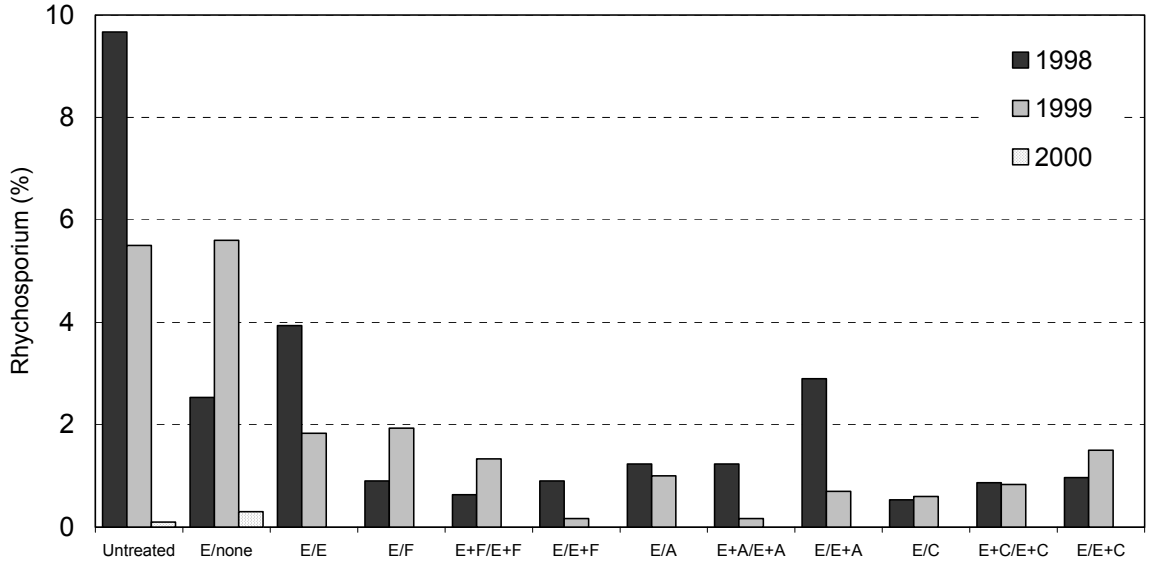
Table 6. Effect of treatments on *R. secalis* infection on leaves 1 and 2, south-west England trials

Tr. no.	Treatment	*Rhynchosporium secalis* infection (%)					
		1998 (GS 71)		1999 (GS 75)		2000 (GS 75)	
		Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2
1	Untreated	6.7	28.3	8.5	43.0	7.3	25.8
2	E/none	4.0	28.3	14.3	37.7	2.9	10.0
3	E/E	1.0	10.0	8.7	27.0	6.8	18.5
4	E/F	1.1	11.3	4.8	18.0	3.8	10.7
5	E+F/E+F	2.1	12.7	5.3	17.2	2.7	10.0
6	E/E+F	1.4	7.0	13.0	35.2	3.7	18.5
7	E/A	2.0	7.0	4.5	21.8	2.6	10.8
8	E+A/E+A	1.7	6.3	5.2	15.5	2.2	13.2
9	E/E+A	2.0	8.0	4.8	21.2	2.7	15.8
10	E/C	1.1	7.3	4.3	23.2	1.8	14.3
11	E+C/E+C	2.1	9.7	4.8	15.0	2.2	10.2
12	E/E+C	4.5	13.0	8.8	25.3	3.4	15.0
L.S.D. (P<0.05)		n/a	11.03	n/a	12.62	3.89	10.12

n/a not applicable: L.S.D. values were only calculated where the effect of treatment was significant (P<0.05).

Fig. 1. Effect of programmes on Rhynchosporium on a) leaf 1 and b) leaf 2, Northern Ireland trials

a)



b)

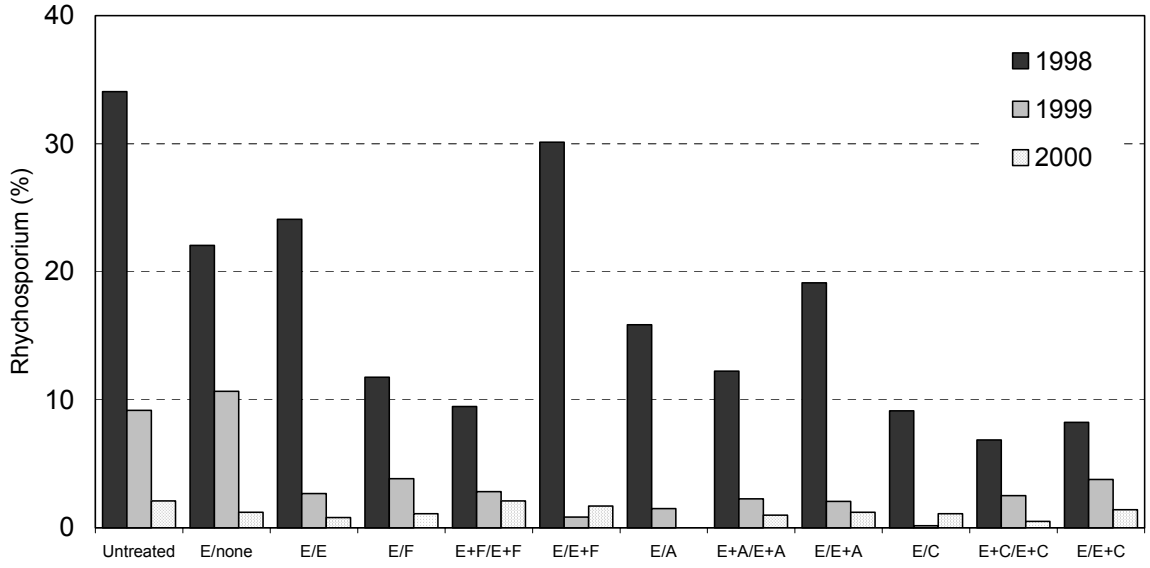
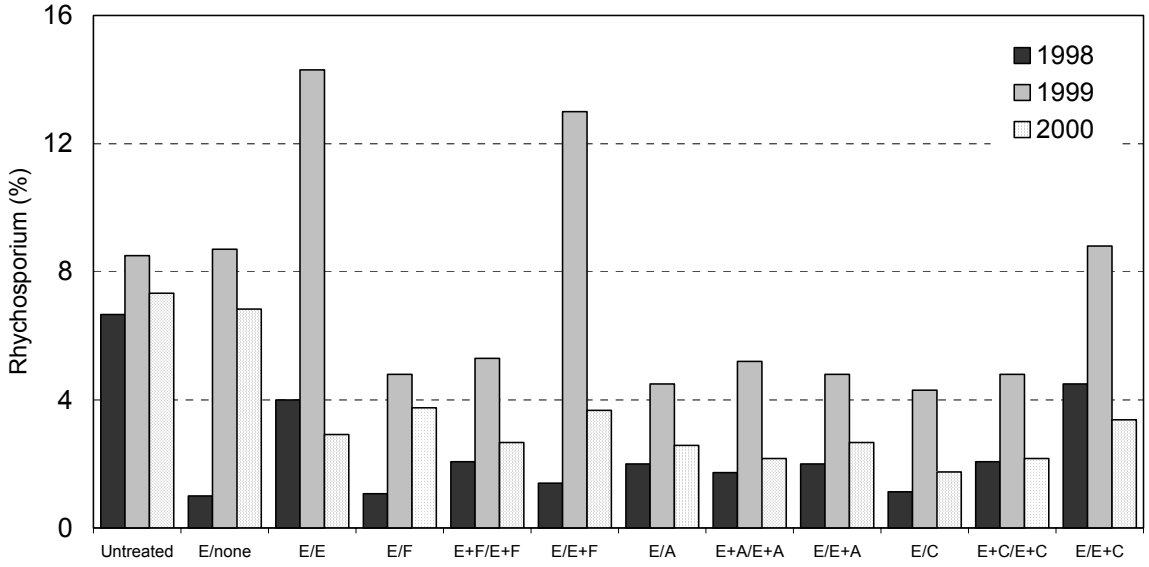


Fig. 2. Effect of programmes on Rhynchosporium on a) leaf 1 and b) leaf 2, South-west England trials

a)



b)

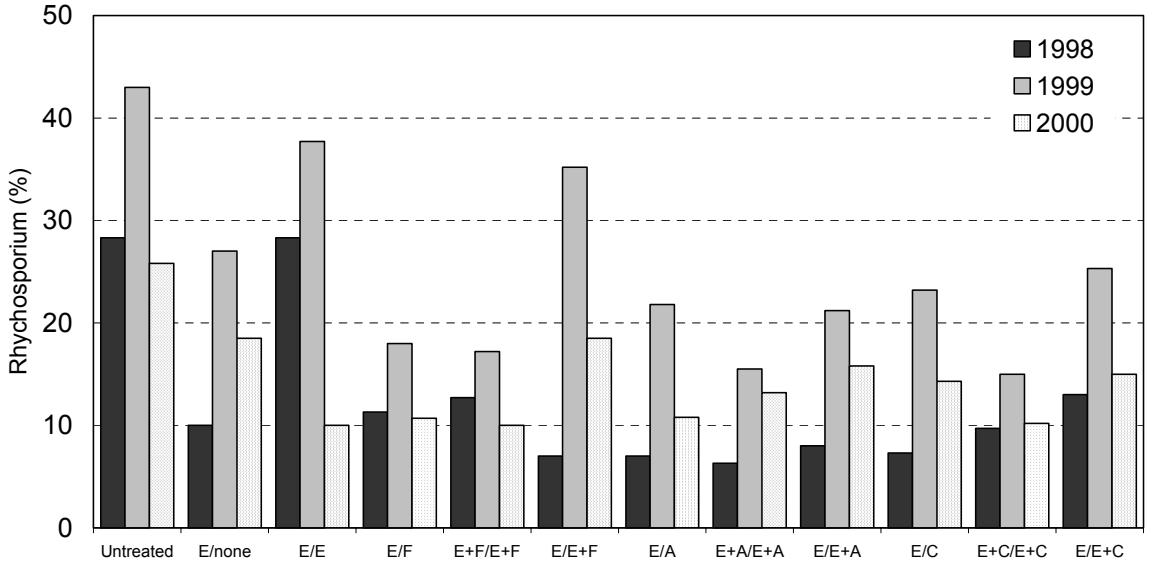
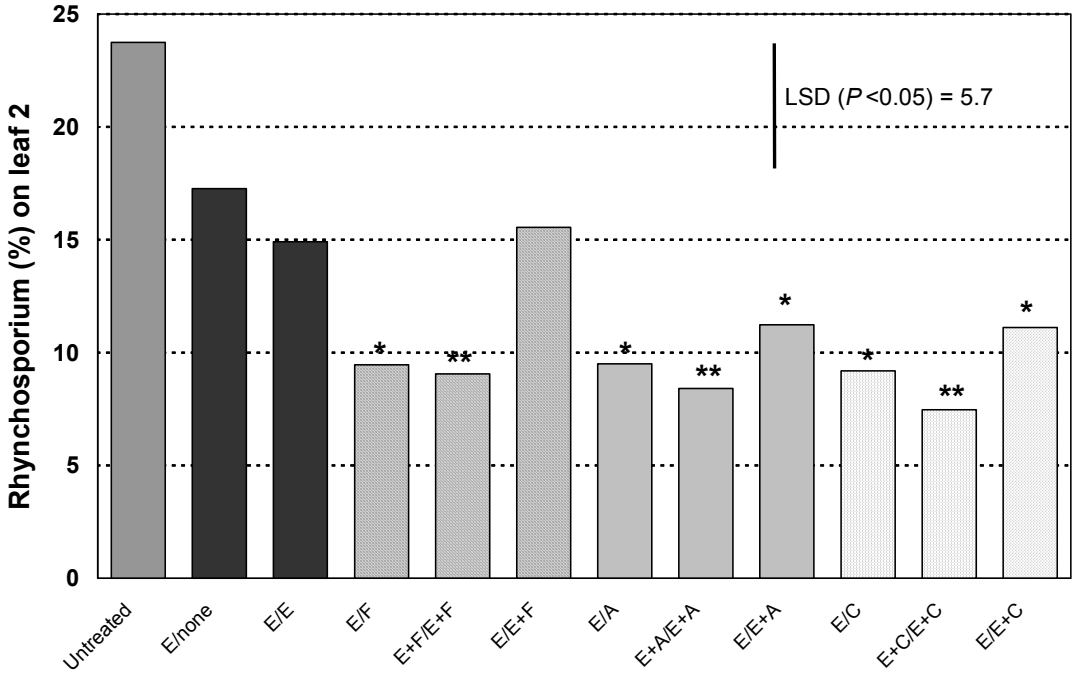
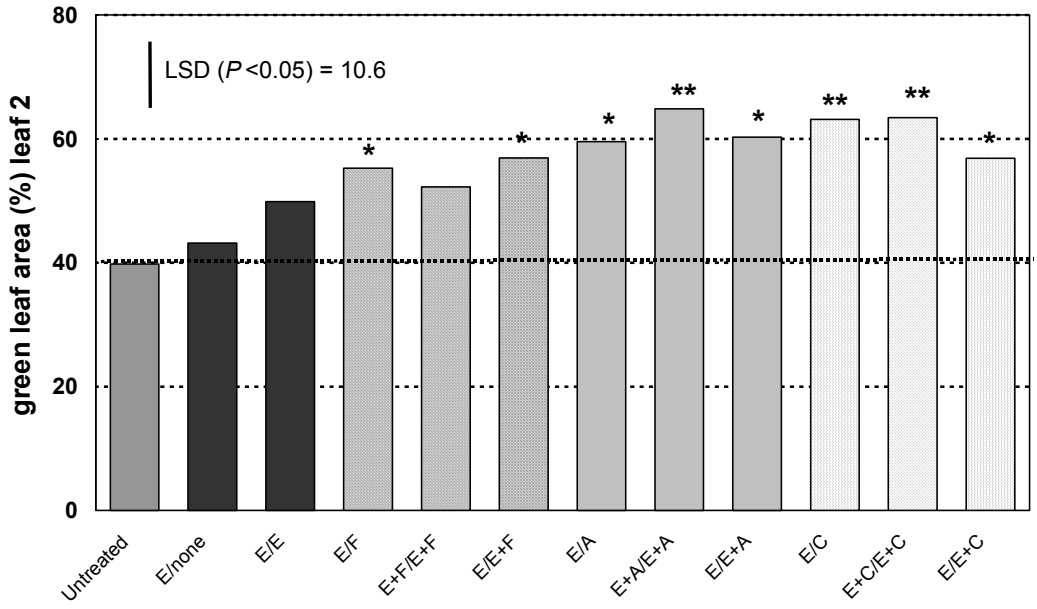


Fig. 3. Overall effect of fungicide programmes on Rhynchosporium on leaf 2, mean of six trials, 1998-2000



All treatments had significantly (P <0.05) less infection than the untreated control
* indicates treatments significantly (P <0.05) less than both the untreated control and the E/none treatment
** indicates treatments significantly (P <0.05) less than untreated control and both the E/none and E/E treatments

Fig. 4. Overall effect of fungicide programmes on green leaf area of leaf 2, mean of six trials, 1998-2000



All treatments except for E/none and E/E were significantly (P <0.05) greater than the untreated control
* indicates treatments significantly (P <0.05) greater than the untreated control and the E/none treatment
** indicates treatments significantly (P <0.05) greater than the untreated control, the E/none treatment and the E/E treatment

Table 7. Effect of treatments on green leaf area on leaf 2, in six trials 1998-2000

Tr. no.	Treatment	Green leaf area (%)					
		Northern Ireland			South-west England		
		1998[a]	1999	2000	1998	1999	2000
1	Untreated	7.2	65.4	93.7	36.7	35.7	0.0
2	E/none	10.1	86.1	97.7	33.3	24.2	7.5
3	E/E	11.5	76.3	95.7	73.0	38.2	4.5
4	E/F	16.8	91.3	97.2	66.7	53.3	6.3
5	E+F/E+F	17.1	89.1	94.9	51.7	56.0	4.7
6	E/E+F	18.0	94.1	96.0	85.0	43.7	4.7
7	E/A	18.6	96.0	100.0	68.3	62.8	11.5
8	E+A/E+A	34.2	93.8	97.4	80.0	68.5	15.2
9	E/E+A	10.0	92.8	97.7	83.0	68.0	10.2
10	E/C	35.0	98.5	97.3	74.0	61.3	12.8
11	E+C/E+C	50.5	91.9	98.5	66.7	66.2	6.8
12	E/E+C	39.5	91.8	97.3	56.7	44.8	11.0
L.S.D. (P<0.05)		20.97	12.5	n/a	30.84	14.59	n/a

a leaf 3 data shown (leaf 2 not assessed)

n/a not applicable: L.S.D. values were only calculated where the effect of treatment was significant (P<0.05).

Effect of fungicide treatments on yield

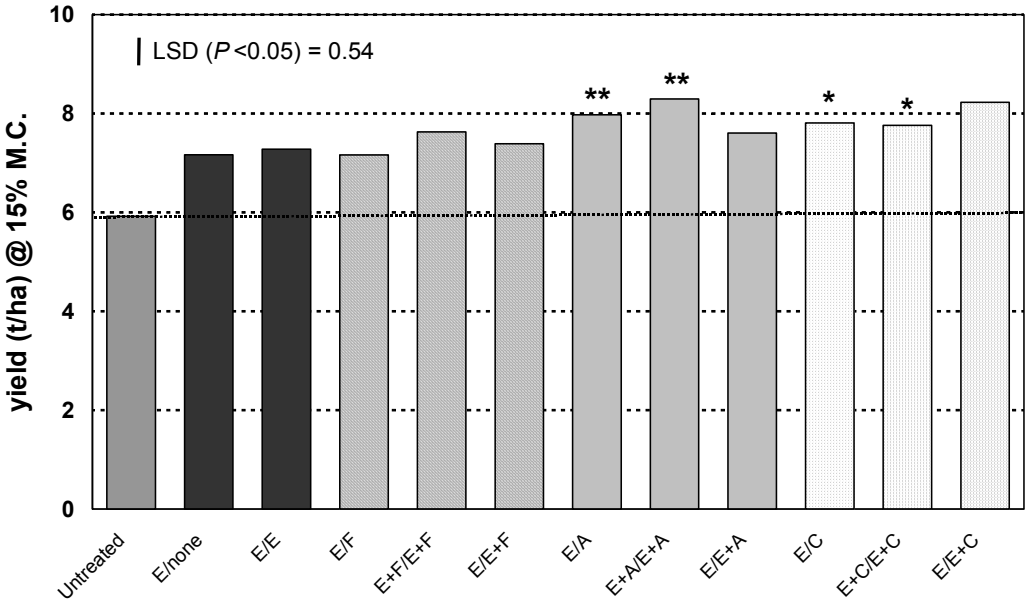
Only the trials in Northern Ireland were planned to be taken to yield, but in 1998 yield data were also obtained from the South-west England trial. In all trials, the lowest yield was produced by the untreated control plots (Table 8). In the overall analysis (Fig. 5), there was a very highly significant effect of treatment ($P<0.001$) and all treatments gave a significantly ($P<0.05$) higher yield than the untreated control. The lowest yielding fungicide treatments were those containing epoxiconazole alone (Tr. 2 and Tr. 3) and the epoxiconazole/fenpropimorph treatment (Tr. 4). Four treatments achieved significantly greater yields than Tr. 2 (epoxiconazole full-rate at T1) and two of these also yielded significantly more than Tr. 3 (half-rate epoxiconazole at T1 and T2). The highest yields were produced by two of the epoxiconazole/azoxystrobin combinations, Tr. 8 (half-rate epoxiconazole + azoxystrobin at T1 and T2) achieving the greatest yield in each of the four trials.

For the three Northern Ireland trials, thousand grain weight and hectolitre weight data were also collected and the overall analyses (Figs. 6a and b) showed a very highly significant ($P<0.001$) effect of treatment ($P<0.001$). All fungicide treatments had significantly ($P<0.05$) greater thousand grain weights and hectolitre weights than did the untreated controls. The same two epoxiconazole/ azoxystrobin combinations, Tr. 8 (half-rate epoxiconazole + azoxystrobin at T1 and T2) and Tr. 7 (full-rate epoxiconazole followed by full-rate azoxystrobin), which achieved the highest yields also had the greatest thousand grain weights and hectolitre weights, which were significantly greater than those from Tr.2 (epoxiconazole full-rate at T1) and Tr. 3 (half-rate epoxiconazole and T1 and T2).

Table 8. Effect of treatments on yield, Northern Ireland trials 1998-2000 and South-west England trial 1998

Tr. no.	Treatment	Yield (t/ha) @ 15% moisture content			
		Northern Ireland			South-west England
		1998	1999	2000	1998
1	Untreated	5.01	6.18	8.66	3.84
2	E/none	5.79	7.25	10.97	4.65
3	E/E	6.06	7.84	10.97	4.25
4	E/F	5.51	8.07	10.58	4.49
5	E+F/E+F	6.82	8.37	10.93	4.39
6	E/E+F	5.94	7.99	11.26	4.35
7	E/A	6.60	8.99	11.33	4.97
8	E+A/E+A	7.29	9.1	11.48	5.30
9	E/E+A	6.35	8.43	11.00	4.64
10	E/C	7.10	8.31	11.15	4.68
11	E+C/E+C	6.62	8.69	11.23	4.50
12	E/E+C	6.63	7.48	11.33	4.43
L.S.D. (P<0.05)		0.908	0.830	0.769	0.388

Fig. 5. Overall effect of fungicide programmes on on yield, mean of four trials, South-west England 1998 amd Northern Ireland 1998-2000



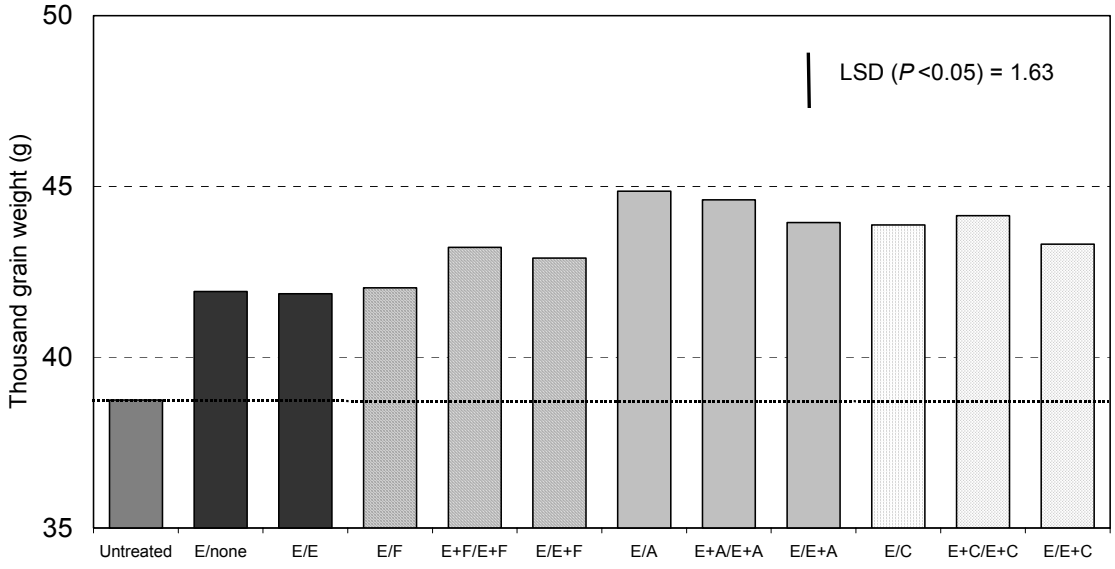
All treatments had significantly (P<0.05) greater yield than the untreated control

* indicates treatments significantly (*P* <0.05) greater than both the untreated control and the E/none treatment

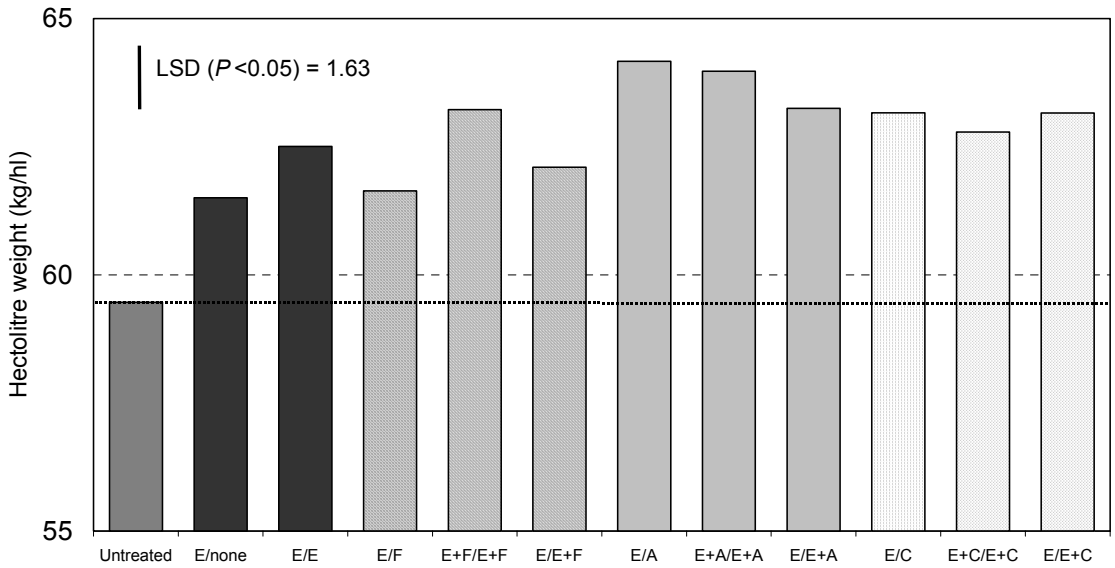
** indicates treatments significantly (*P* <0.05) greater than untreated control and both the E/none and E/E treatments

Fig. 6. Overall effect of fungicide programmes on a) thousand grain weight and b) hectolitre weight, mean of three Northern Ireland trials, 1998-2000

a)



b)



Sensitivity to epoxiconazole

Isolation of *R. secalis* from leaf blotch samples collected from the trials proved very problematic. It was essential to delay sample collection until some time after the second fungicide application in order to permit any effect of treatment on sensitivity to be expressed, but by this stage leaves were often colonised by other organisms which interfered with isolation process and overgrew the slow-growing *R. secalis*. The procedure was improved in the year 2000 by the addition of iprodione to suppress other fungi (using results obtained in an HGCA-funded undergraduate bursary study). However, in 1999 insufficient isolates were obtained from either the Northern Ireland or the South-west England trials to permit comparisons between treatments.

In 1998, over 300 isolates were obtained from the Northern Ireland trial and over 200 from the South-west England trial and tested for epoxiconazole sensitivity using the ELISA plate method. In 2000, just under 200 isolates were obtained from the Northern Ireland trial and over 130 isolates from the South-west England trial.

Northern Ireland trial 1998

Results were expressed as both ED_{50} and MIC values for this trial to ascertain if the different methods of expressing sensitivity affected the apparent influence of treatments. The results shown in Fig. 7a (ED_{50} values) and Fig. 7b (MIC values) demonstrate very similar trends regardless of which sensitivity measure is used. The mean pre-treatment MIC value (0.15 mg epoxiconazole/l) was substantially lower than the post-treatment mean across all treatments (1.04 mg/l) indicating that treatment had selected for less sensitive isolates. The least sensitive isolates were associated with two applications of half-rate epoxiconazole alone (Tr. 3). The other programmes had fewer of the least sensitive isolates, but there were no consistent differences between them.

South-west England 1998

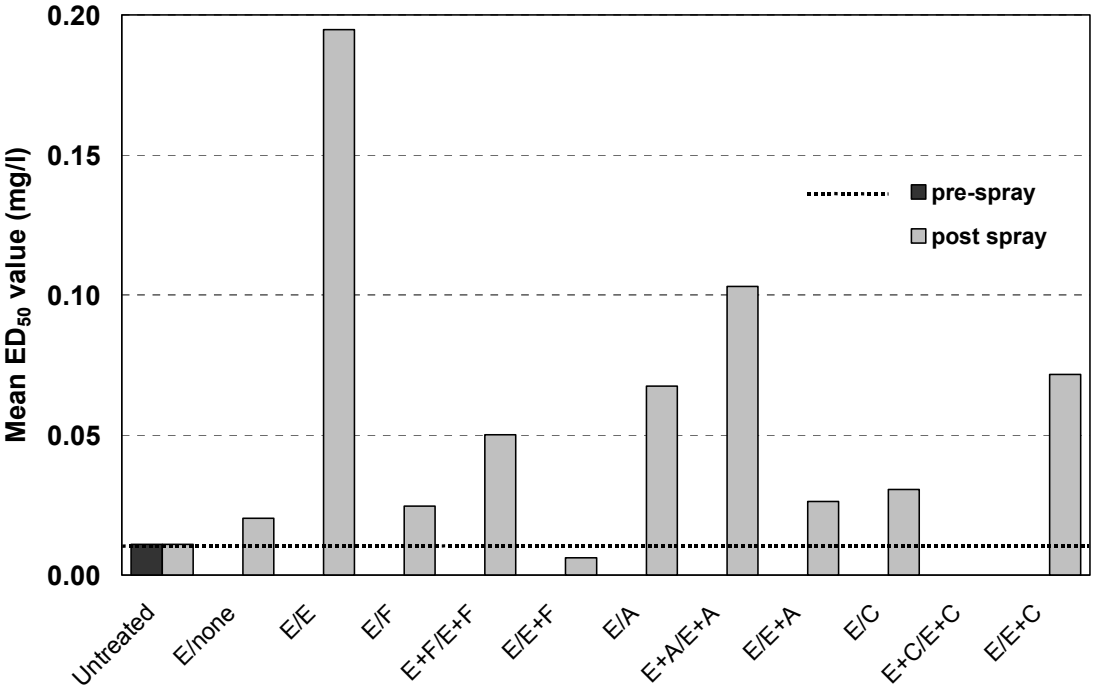
In 1998, only five pre-treatment isolates were successfully tested, which was considered insufficient to give a reliable base-line sensitivity for this trial. As with the Northern Ireland trial, the least sensitive isolates were associated with Tr. 3 (two applications of half-rate epoxiconazole alone), but the differences between the fungicide programmes were less than in Northern Ireland and were not significant (Fig. 8).

Northern Ireland 2000

There was a decrease in sensitivity from a mean MIC value of 0.58 mg epoxiconazole/l before treatment to an overall mean of 1.98 mg/l after treatment, but the effect of the different fungicide programmes showed no clear trend (Fig. 9) and there were no significant differences between them.

Fig. 7. Effect of fungicide programmes on sensitivity of *Rhynchosporium secalis* to epoxiconazole in the Northern Ireland field trial, 1998 as indicated by a) ED_{50} and b) MIC values

a)



b)

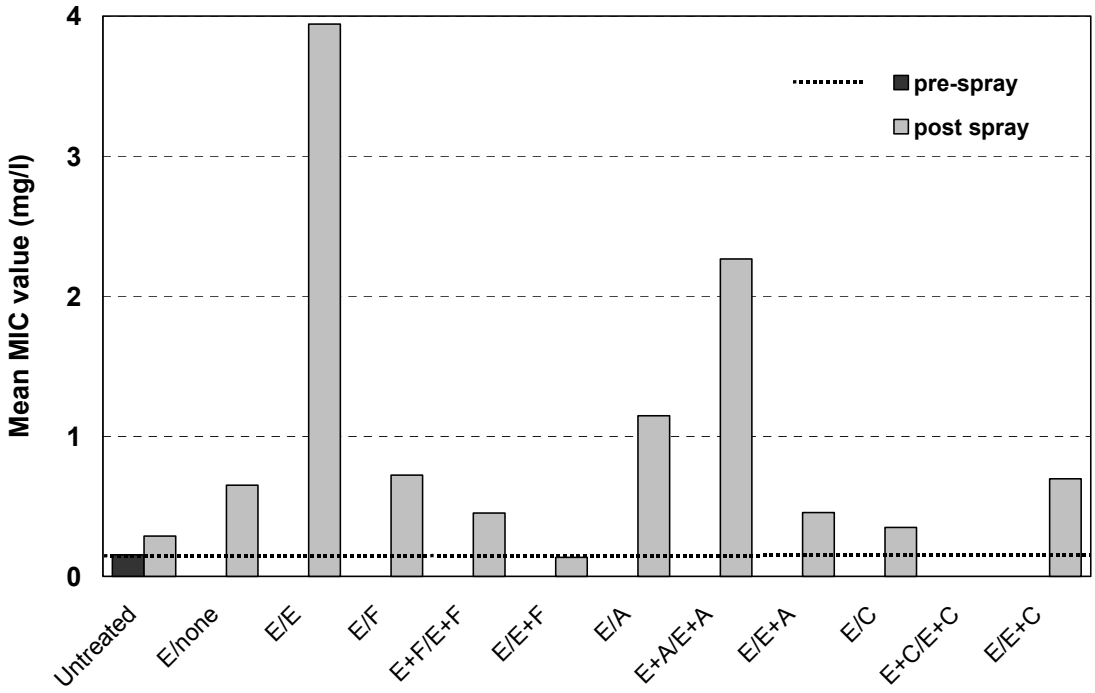


Fig. 8. Effect of fungicide programmes on sensitivity of *Rhynchosporium secalis* to epoxiconazole in the South-west England field trial, 1998

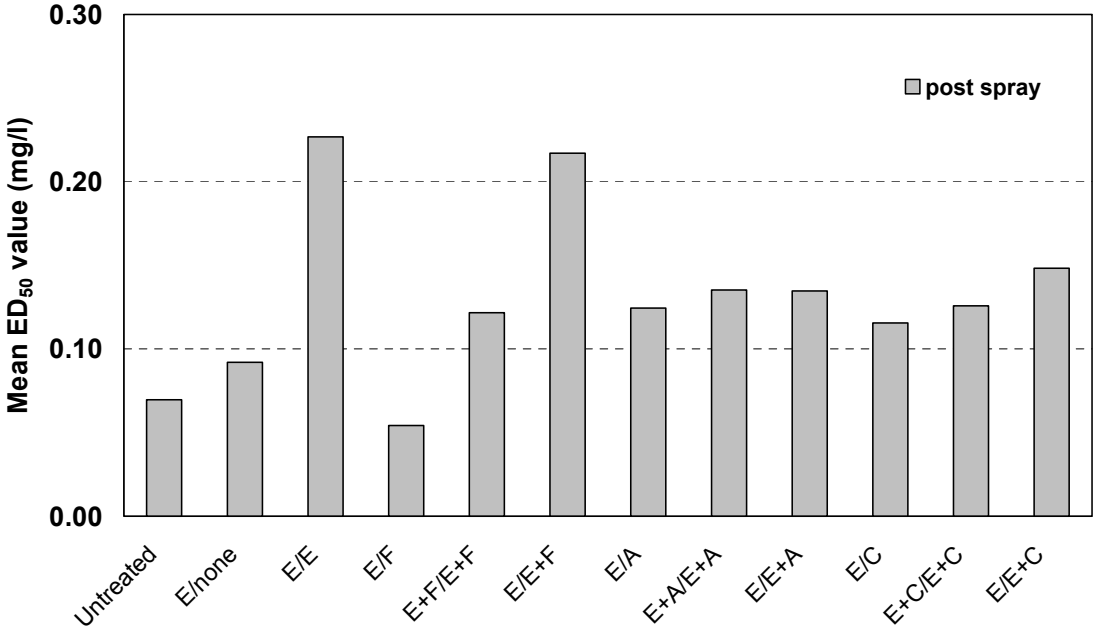
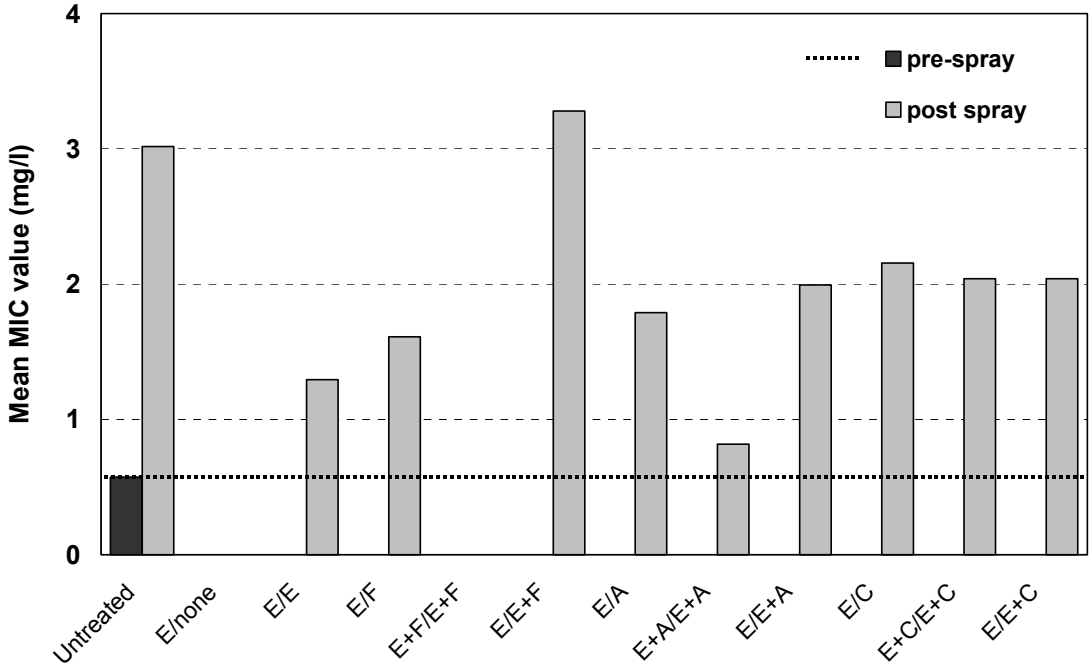


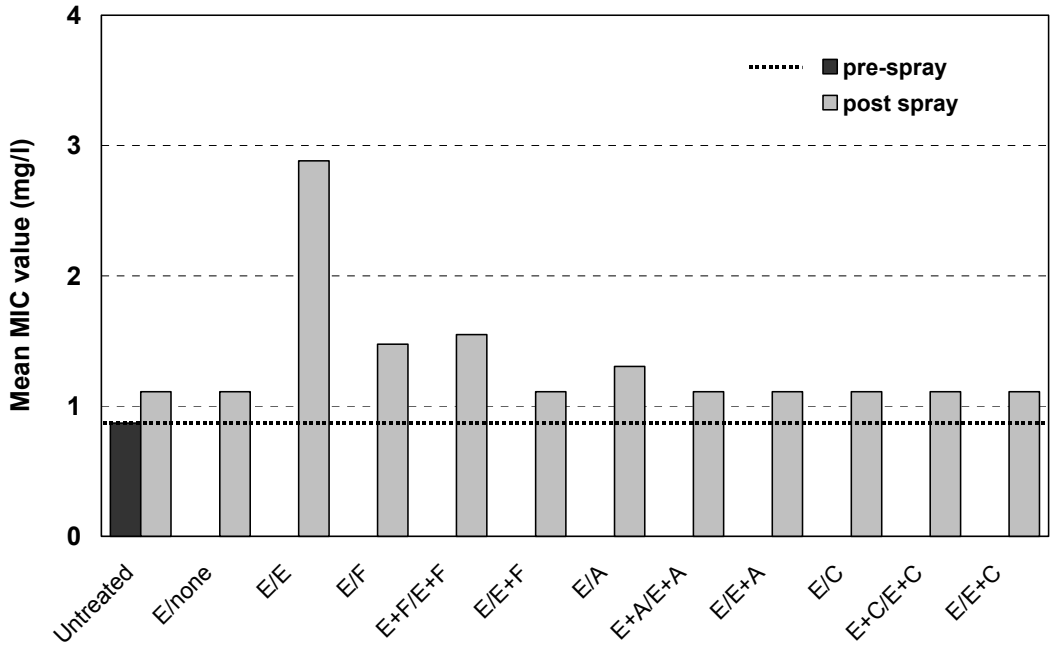
Fig. 9. Effect of fungicide programmes on sensitivity of *Rhynchosporium secalis* to epoxiconazole in the Northern Ireland field trial, 2000



South-west England 2000

The results depicted in Fig. 10 show that sensitivity to epoxiconazole decreased from a mean MIC value of 0.87 mg/l before treatment to an overall mean of 1.23 mg/l following fungicide application, but only to any marked extent in the plots which received two half-rate applications of epoxiconazole alone (Tr. 3).

Fig. 10. Effect of fungicide programmes on sensitivity of *Rhynchosporium secalis* to epoxiconazole in the South-west England field trial, 2000



Sensitivity trends across the four trials

In three out of the four trials, sensitivity was lower in *R. secalis* isolates collected after fungicide treatments were applied than in those collected before treatment. Also in three out of four trials, the least sensitive isolates came from the plots which had received two half-rate applications of epoxiconazole (Tr. 3). In contrast, there was no evidence that a single full-rate epoxiconazole application (Tr. 2) had a greater tendency to select for resistant fungicide strains than the other fungicide programmes. There was no consistent difference in effect on epoxiconazole sensitivity between the three partner fungicides, but all tended to reduce selection for resistance compared with the application of two half-rate applications of epoxiconazole alone.

DISCUSSION

To achieve consistent and sustainable control of a pathogen such as *R. secalis*, where the key fungicides are single-site inhibitors and the pathogen is known to adapt to fungicide use by the selection of resistant strains, both effective disease control and resistance management must be considered.

The DMI fungicides have been the key element of control programmes for *R. secalis* since the 1980s, but their effectiveness has gradually been eroded by selection of less sensitive strains. However, selection for resistance by DMIs within field trials has proved difficult to demonstrate with many pathogens because of the quantitative nature of resistance, which results in gradual shifts in sensitivity rather than an abrupt complete loss of response to the fungicide as can occur with e.g. the benzimidazoles.

The barley disease surveys of 1997 -2001 have estimated the losses in England and Wales due to the residual levels of Rhynchosporium after fungicide treatments as (£m/annum) 3.23, 6.65, 4.07, 5.43 and 2.59, a total of nearly £22m in 5 year period (N V Hardwick, personal communication). This is with most farmers applying relatively effective control measures; the situation will become much worse if fungicide efficacy declines further and ineffective treatments are applied. Disease pressure from Rhynchosporium remains high, indicating that fungicides remain a principal method of control over varietal resistance. This is in contrast to mildew and net blotch which have both dropped in importance in recent years at least in part due to the availability of barley cultivars with effective resistance. This makes Rhynchosporium currently the most economically important disease of barley.

The series of trials reported here have unequivocally demonstrated that the DMI epoxiconazole, previously considered the DMI most active against *R. secalis*, can no longer be relied on to give effective control if used alone. Indeed, in some of the trials epoxiconazole alone had little or no effect in reducing Rhynchosporium or increasing green leaf area. However, despite this limited effect in controlling disease, programmes based on epoxiconazole did give significant yield benefits.

The three partner fungicides evaluated all improved disease control and yield. The programmes based on two applications of half-rate epoxiconazole with half-rate azoxystrobin or cyprodinil performed best overall, cyprodinil being marginally more effective in terms of disease control, while azoxystrobin combinations achieved the best yields.

In terms of effects of fungicide treatment on sensitivity of *R. secalis* to epoxiconazole, the trials demonstrate that even within a single season, fungicide treatment selects for less sensitive pathogen strains. This selection was most marked where two applications of half-rate epoxiconazole were applied. In contrast, a single full-rate epoxiconazole application had a lesser effect, but cannot be advised in view of its relatively poor performance in controlling the disease. The use of a partner fungicide reduced the selection of resistant

strains compared with applying epoxiconazole alone, but none of the three evaluated completely prevented the tendency for DMI sensitivity to be lower after fungicide treatment.

Comparison of the DMI sensitivities observed within these trials with those found in previous studies of *R. secalis* sensitivity in the UK are complicated by the fact that in the earlier work, most of the sensitivity testing was against the DMI triadimenol and although some other DMIs were included, epoxiconazole was not used since this was not then available. It was not feasible to use triadimenol in the present study, since, although during the period 1975-1981 the mean MIC value for *R. secalis* isolates against triadimenol was 0.9 mg/l, by the mid 1990s, the majority of isolates had MIC values for triadimenol of >50 mg/l (Hollomon, 1997) and the use of higher concentrations is limited by solubility. Epoxiconazole is inherently much more active against *R. secalis*, but even within the time period of this study, it was observed that the number of the less sensitive isolates (MIC values of 3 mg/l or more) was greater in the year 2000 than in 1998. In continuing studies of epoxiconazole sensitivity (as part of HGCA Project No. 2322), greater proportions of less sensitive isolates with MIC values for epoxiconazole of 10 mg/l or more have been obtained from trial sites in Northern Ireland and Scotland.

This situation contrasts with that found for *Septoria tritici* on wheat where a recent HGCA-funded project (no. 1406) showed that, despite the wide range of sensitivities to epoxiconazole found in this pathogen, there were no overall changes in sensitivity during the course of the study (Hollomon *et al.*, 2002).

It is concluded that:

- selection for DMI resistance is continuing to occur in *R. secalis.*
- with the range of pathogen sensitivities found in the present study, a DMI such as epoxiconazole is a useful component of a control programme for *R. secalis*, but must be supplemented by a partner fungicide.
- fungicides with different modes of action from the DMIs from the strobilurin (QoI) and anilinopyrimidine group are the most effective partners in terms of disease control and yield.
- use of a partner fungicide helps to reduce selection for DMI resistance, but cannot prevent it.

FUTURE

While DMI use continues, DMI sensitivity will continue to decline, although the effective life of these fungicides can be prolonged by avoiding their use as sole active ingredients and particularly by avoiding repeated use of half-rates of DMIs alone. In some regions, DMIs may no longer be worthwhile components

of *R. secalis* control programmes, but it is imperative that sensitivity is assessed before assuming that their use is not justified, since reliance on strobilurin or anilinopyrimidine fungicides alone in itself poses potential risks of resistance.

ACKNOWLEDGEMENTS

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OUTPUT AND TECHNOLOGY TRANSFER

Scientific publications

Cooke, L.R., Locke, T., Lockley, K.D., Phillips, A.N., Sadiq, M.D.S., Coll, R., Black, L., Taggart, P.J. & Mercer, P.C. (2001). DMI sensitivity in *Rhynchosporium secalis* as influenced by fungicide programmes. Poster with abstract presented at Resistance 2001, Rothamsted, 24-26 September.

Cooke, L.R., Coll, R., Black, L., Taggart, P.J., Mercer, P.C., Locke, T., Lockley, K.D., Phillips, A.N. & Sadiq, M.D.S. (2001). DMI sensitivity in *Rhynchosporium secalis* as influenced by fungicide programmes. *Resistant Pest Management Newsletter* **11**, no. 2 (http://whalonlab.msu.edu/rpmnews/vol.11_no.2/abstracts/rpm_abstracts.htm).

Technical publications and contributions

HGCA Topic Sheet no. 42 Rhynchosporium control programmes, February 2001.

Crops magazine, 3 February 2001 'New tactics to tackle rhyncho', article by Sarah Henly.

Farmers Weekly, 2 March 2001, 'Seeing off rhyncho'

HGCA Press release 10 April 2001, 'Change fungicide tactics to combat rhynchosporium'.

Input into *"Guidelines for preventing and managing fungicide resistance in cereal pathogens"* (2000) and "*Fungicide resistance"* (2001) produced by FRAG-UK and the HGCA.

Presentations

Cooke, L.R.; Taggart, P.J. and Mercer, P.C. (1998). Managing fungicide resistance in Septoria and Rhynchosporium. HGCA Agronomy Roadshow '98, Greenmount College, Antrim, Northern Ireland, pp. 7.1-7.6.

Cooke, L.R. (1998). Managing fungicide resistance in cereal pathogens. Interview on Radio Ulster's *Farmgate* programme, 25 November.

Presentations have also been made for the Agricultural Research Institute for Northern Ireland Open Day (July 2000) and the Ulster Arable Society visit to the Agricultural & Food Science Centre, Newforge (December 2000)

DARD Arable Crop Advisers have been briefed annually on Northern Ireland results.

Data were used at the UK Recommended List Trials Cereal Fungicide Programme Selection Meetings.