Project Report No. 436

August 2008

Price: £7.00

Understanding fungicide mixtures to control Rhynchosporium in barley and minimise resistance shifts

by

S J P Oxley[1], F Burnett[1], E A Hunter[2], B A Fraaije[3], L R Cooke[4], P C Mercer[4] and A Gilchrist[5]

[1]SAC Commercial Ltd., West Mains Road, Edinburgh EH9 3JG
[2] Biomathematics and Statistics Scotland (BioSS), The University of Edinburgh, JCMB, The King's Buildings, Edinburgh, EH9 3JZ
[3] Rothamsted Research, West Common, Harpenden, Herts. AL5 2JQ
[4] Applied Plant Science & Biometrics Division, Agri-Food & Biosciences Institute (AFBI), Newforge Lane, Belfast, Northern Ireland, BT9 5PX
[5] Scottish Agronomy Ltd, Arlary Farm, Milnathort, KINROSS, KY13 9SJ

This is the final report of an HGCA project that ran for forty-five months from July 2004. The project was sponsored by SEERAD (£398,087) and HGCA (£164,214, Project No. 3028) making a total of £562,301.

Contents

Abstract 1

Summary 2

Technical Report 7

Contribution of individual fungicides to rhynchosporium control in mixtures 7
Introduction 7
Materials and Methods 9
  Field trials 9
  Disease assessments in field 12
  Harvest assessments and economic response to treatment 13
Results 21
  Crop yields 22
  Specific weights 24
  Margin over fungicide costs (£/ha) 26
  Eradicant activity against rhynchosporium 28 days after treatment 40
  Rhynchosporium eradication in high disease pressure situations 43
  Use of diagnostics to forecast disease pressure 47
  Impact of fungicide programmes based on late-season levels of DNA 52
  Green leaf area retention 55
Discussion 61

Impact of fungicide mixtures and sequences on rhynchosporium sensitivity ... 66
Introduction 66
  Literature review 66
  Aims 68
Materials and Methods 70
Results 72
  R. secalis sensitivity to fungicides 72
  Cross-resistance 75
  Variation by year 78
  Variation by site 82
  Variation by treatment 86
Discussion 90

Overall Conclusions 91

References 93

Abstract

The objective was to investigate the impact of fungicides used in mixtures and sequences of barley in terms of eradicant and protectant activity against rhynchosporium leaf scald (rhynchosporium) caused by *Rhynchosporium secalis*. Since resistance to several fungicide groups is becoming a major problem, the research also tested for potential resistance shifts to ensure that the most effective fungicide mixtures did not increase resistance. New molecular techniques were used to identify the presence of *R secalis* prior to symptom development to determine their practical use as a guide to disease risk.

Treatment with a single fungicide did not achieve the best disease control, yield or margin. Prothioconazole (Proline) was the key fungicide component in a fungicide mixture for both disease control and yield in winter and spring barley. Cyprodinil (Unix) was also key for yield in winter barley, but less important in spring barley. Pyraclostrobin (Vivid) was an important component of a mixture where rhynchosporium eradication was required. Chlorothalonil (Bravo) was a useful mixing partner, but in two-way mixtures, rhynchosporium eradication was reduced where the dose ratio was 1:1. This effect was not seen in a three-way mixture where the dose ratio of chlorothalonil to other fungicides was 0.5:1.

Using prothioconazole alone shifted the rhynchosporium population towards greater resistance compared to using the fungicide in mixture with a second active ingredient. Mixtures therefore will limit the increase in resistance occurring. Prothioconazole provided good control of rhynchosporium in these situations, but pyraclostrobin and cyprodinil also gave favourable disease control.

At grain prices of £175/tonne, two-way fungicide mixtures were the most cost effective approach for spring barley and three-way mixtures for winter barley. At lower grain prices of £75/tonne, two-way mixtures were the most cost effective for both.

Fungicide diagnostics were a useful tool to determine disease levels in high pressure crops by testing leaves and shoots before treatment. Visual assessments were effective, but a diagnostic test was more sensitive where disease symptoms had yet to appear. By testing rhynchosporium levels late in the season, it can be concluded that a yield response to fungicide occurs both in crops where visual symptoms are present and also where rhynchosporium DNA levels were high in the absence of symptoms. The lowest yield responses occurred where DNA levels and symptoms were low in the upper leaves. Plant breeders will need to redefine a resistant variety as one where visual symptoms are not present and where the fungus cannot be detected inside the plant. These results suggest varieties can respond to fungicide in the absence of visual disease symptoms but where the fungus is detectable at 10-40 pg DNA inside symptom free plants.

Summary

Rhynchosporium leaf scald (rhynchosporium) is a major wet weather disease in winter and spring barley caused by the fungus *Rhynchosporium secalis,* which can lead to extensive leaf death causing losses in yield. The aim for a successful grower is to achieve optimum yield, quality, and margin from a crop of barley. Crop disease will impact on this and varietal resistance alone is insufficient to deal with rhynchosporium.

Barley seed is a key source of *R secalis* alongside spores in barley trash, volunteers, rain splash spores and potentially airborne spores (Zhan *et al*. 2008). Rhynchosporium symptoms can develop in the autumn both on barley volunteers and in the crop, but widespread infection develops in January to February as a consequence of seed infection. It is common for disease symptoms to be present in a crop at the time of fungicide treatment, so effective disease eradication is required with fungicides. A typical timing for the first fungicide in winter barley is at stem extension (GS31-32). In high disease pressure situations this may be too late and earlier treatments are recommended at GS25-30 in the spring. Established disease in the winter may also warrant fungicide action with fungicides in exceptional situations (Oxley & Burnett 2008).

Spring barley sown in the winter is likely to follow disease patterns observed in winter barley, but spring barley sown in March to April will grow rapidly leading to a situation where no rhynchosporium symptoms are present at the time a fungicide treatment is applied at mid to late tillering (GS25-30). In this situation, fungicides are required to protect the crop from disease – a scenario which is more successful for most fungicides compared to attempts to eradicate established disease.

In the absence of robust varietal resistance for high disease pressure regions, fungicides play an important role in disease management and this research aims to understand how to use fungicides in mixtures to achieve effective eradication, protection, yield and margin.

One aspect of fungicide use which is of less immediate interest to a grower is fungicide resistance. For a grower, short term gains through using a particular fungicide programme may override the longer term risk of a build up in fungicide

resistance. This research looks at the impact fungicide mixtures have on resistance build up and looks at programmes which may achieve both the growers aims and minimise the risk of a build up in resistance.

Different growth and disease development patterns mean the management of rhynchosporium in winter barley requires a different approach to the spring crop. Relying on visual symptoms as a trigger to treat a crop with fungicide will mean disease is already well established and potentially causing damage to yield. New diagnostics can help identify *R secalis* DNA both in seed and in symptom-free plants. Part of this research therefore looked at the potential to use diagnostics as part of a decision process to treat a crop before disease symptoms were visible.

Effective fungicide mixtures to control rhynchosporium and achieve yield

Eradication of rhynchosporium is a greater challenge for fungicide mixtures than protection. No individual fungicide was sufficiently effective to be used alone either for disease control or optimum yields. The key components of a mixture under these circumstances were pyraclostrobin (Vivid) and prothioconazole (Proline). Fungicide mixtures to avoid for eradication include most two-way mixtures where chlorothalonil (Bravo) was a component (e.g. chlorothalonil + pyraclostrobin, chlorothalonil + cyprodinil, chlorothalonil + fenpropimorph). This negative effect on disease eradication was less of an issue in the two-way mixture with prothioconazole and in three-way mixtures where there was a higher dose of alternative fungicides.

Rhynchosporium protection was straightforward and all two and three-way mixtures achieved good protection. Some mixtures did however increase yield and margin more than others, so this should be taken account of in choosing mixtures (see summary below).

Increasing the components in a mixture led to an increase in yield. For winter barley at £175/tonne, three-way mixtures were the most cost effective. At grain prices of £75/tonne and for spring barley, two-way mixtures were the most cost effective.

Active ingredients use in mixtures

	Active ingredients g/ha		
Code	**Active ingredient 1**	**Active ingredient 2**	**Active ingredient 3**
PC	prothioconazole 100	fenpropimorph 375	-
PV	prothioconazole 100	pyraclostrobin 125	-
PU	prothioconazole 100	cyprodinil 300	-
PB	prothioconazole 100	chlorothalonil 500	-
CV	fenpropimorph 375	pyraclostrobin 125	-
CU	fenpropimorph 375	cyprodinil 300	-
CB	fenpropimorph 375	chlorothalonil 500	-
VU	pyraclostrobin 125	cyprodinil 300	-
VB	pyraclostrobin 125	chlorothalonil 500	-
UB	cyprodinil 300	chlorothalonil 500	-
PCV	prothioconazole 100	fenpropimorph 375	pyraclostrobin 125
PCU	prothioconazole 100	fenpropimorph 375	cyprodinil 300
PCB	prothioconazole 100	fenpropimorph 375	chlorothalonil 500
PVU	prothioconazole 100	pyraclostrobin 125	cyprodinil 300
PVB	prothioconazole 100	pyraclostrobin 125	chlorothalonil 500
PUB	prothioconazole 100	cyprodinil 300	chlorothalonil 500
VCU	pyraclostrobin 125	fenpropimorph 375	cyprodinil 300
VCB	pyraclostrobin 125	fenpropimorph 375	chlorothalonil 500
VUB	pyraclostrobin 125	cyprodinil 300	chlorothalonil 500
UCB	cyprodinil 300	fenpropimorph 375	chlorothalonil 500

Effective mixtures for rhynchosporium control and yield

Code	Rhynchosporium eradication	Rhynchosporium protection	Yield & value (winter barley)	Yield & value (spring barley)
PC	++	+++	+++	++
PV	+++	+++	+++	+++
PU	+++	+++	+++	+++
PB	+++	+++	+++	++
CV	++	+++	+	++
CU	+++	+++	+	++
CB	+	+++	+	++
VU	+++	+++	+	++
VB	+	+++	++	++
UB	+	+++	+++	++
PCV	+++	+++	+++	++
PCU	+++	+++	+++	+++
PCB	++	+++	+++	+++
PVU	+++	+++	+++	+++
PVB	+++	+++	+++	+++
PUB	+++	+++	+++	+++
VCU	+++	+++	++	+++
VCB	+++	+++	+++	++
VUB	+++	+++	++	+++
UCB	++	+++	++	+

Good	+++
Average	++
Poor	+

Fungicide resistance in *Rhynchosporium secalis*

There is a wide range of sensitivity to triazole fungicides (epoxiconazole and prothioconazole). This suggests there are populations of *Rhynchosporium secalis* which are resistant to both these key barley fungicides. There was a significant correlation between the sensitivities of isolates to epoxiconazole and to prothioconazole. This shows that using either of these fungicides will also increase resistance to the other.

The majority of *Rhynchosporium secalis* isolates were sensitive to the strobilurin fungicides pyraclostrobin and fluoxastrobin and they fell within a narrow band of sensitivity. Some isolates appeared less sensitive and tests will be done to see if this is a real effect. It is suggested that this is an artefact since there was no correlation between the sensitivities of these isolates to the two strobilurin fungicides. It can be concluded therefore that *Rhynchosporium secalis* remains highly sensitive to this group of fungicides.

Rhynchosporium secalis isolates were generally very sensitive to cyprodinil (Unix). Some isolates were outside this range however and were more resistant. Fewer isolates were tested against fenpropimorph (Corbel) than for other fungicides. Most were within a narrow band, but a few isolates were less sensitive.

Rhynchosporium secalis sensitivity ranged widely between sites, but no drift in sensitivity was seen between the years. The greatest effect between sites was observed with the triazole fungicides. *R. secalis* was more resistant to epoxiconazole in the north of Scotland on winter barley compared to the South Scotland or Northern Ireland.

Using prothioconazole alone caused the biggest shift in resistance during the season. This was not the case where prothioconazole was applied in a two-way mixture with chlorothalonil, cyprodinil, pyraclostrobin or fluoxastrobin (data not shown) or fenpropimorph. Sensitivity data from three-way mixtures are limited due to the effective control of disease, but it can be assumed three-way mixtures will behave similarly to the two-way mixtures.

In conclusion, the biggest concern in resistance is with triazole fungicides. There is evidence that using one will lead to an increase in resistance of another. Use of

prothioconazole alone can increase resistance within a season, but use of prothioconazole in a two-way mixture will stop this effect. Prothioconazole must always be used in a mixture as an effective anti-resistance strategy.

Diagnostics as an aid to disease risk

Rhynchosporium DNA can be detected in the leaves, shoots and stems of barley before symptoms appear. DNA levels were higher in winter barley compared to spring barley where the subsequent level of symptoms was also higher. Weather plays an important part in disease infection and in the three seasons of trials, higher disease pressures occurred in a wet spring as opposed to a dry spring. DNA levels alone are therefore an insufficient trigger to determine a high risk crop. Diagnostics were as effective as visual assessment to determine the potential high risk of an outbreak. Diagnostics are however more sensitive than visual assessment at the early stages of an epidemic before symptoms appear. Since seed is known to be an important source of infection, testing leaves and shoots over the winter will be a useful guide to the crops with the greatest risk of disease developing. This information will be used in risk decision tools currently being developed in Scottish Government funded research.

Importance of asymptomatic infections

The detection of *Rhynchosporium secalis* DNA inside plants which show no symptoms leads to the question of the relative importance of symptom versus symptomless infection. To address this question, trials were categorised into high and low visual disease late in the season (based on spring rainfall) and high and low DNA levels at the end of the season. Where visual symptoms were high, yield responses to fungicide were also high. However, the same yield response was seen where symptoms were low, but *R secalis* DNA levels were high in the leaves. This observation requires further study, but if the effect is consistent, future advice on late fungicide use may be based upon the level of DNA in the upper leaves to determine risk of yield loss from disease.

Technical Report

Contribution of individual fungicides to rhynchosporium control in mixtures

Introduction

HGCA fungicide performance research on barley investigates the efficacy of existing and new fungicides in high disease pressure situations. The data produced are useful to assist growers understand the relative benefits of protectant and eradicant activity of individual fungicides, but they fall short of assisting growers on how to understand the principles of using fungicides in mixtures and sequences and their impact on resistance shifts.

In a previous study on rhynchosporium in barley (Oxley *et al.* 2003), aspects concerning the sensitivity of rhynchosporium to triazole fungicides and the efficacy of fungicide mixtures were investigated and a start was made on examining the relative contributions made by individual fungicides applied in mixtures to rhynchosporium protection and eradication, and to crop yield and quality. It was clear from this earlier research that for effective control of rhynchosporium, growers are reliant upon a limited number of effective fungicides. Practical advice based on this research is available in an annually updated Technical Note (Oxley & Burnett 2008)

Strobilurin fungicides and triazole fungicides form key components of mixtures. The fungicide groups addressed in this report are described in Table 1. The Fungicide Resistance Action Committee (FRAC) categorises the risk of resistance as low for chlorothalonil (Bravo), medium for prothioconazole (Proline), fenpropimorph (Corbel), cyprodinil (Unix) and high for the Quinone outside Inhibitor (QoI) fungicide pyraclostrobin (Vivid). With recent changes in resistance to QoI fungicides in other pathogens, there was an urgent need to re-visit mixtures to manage rhynchosporium, focussing on those where there is no strobilurin component and also quantifying potential resistance shifts to other major barley fungicides when used in mixtures. Reliance on the triazole fungicide prothioconazole also causes concern, since the activity of other triazoles (triadimenol, flusilazole and epoxiconazole) has declined since their introduction (Kendal *et al* 1993).

Table 1 Fungicides used in the study

Trade name	Code	Active Ingredient	Group name	Resistance risk	FRAC code
Proline	P	prothioconazole	Demethylation inhibitor (DMI)	Medium	3
Corbel	C	fenpropimorph	Morpholine	Low to medium	5
Vivid	V	Pyraclostrobin	Quinone outside Inhibitor (QoI)	High	11
Unix	U	Cyprodinil	Anilino pyrimidine (AP)	Medium	9
Bravo	B	Chlorothalonil	chloronitrile	Low	M5

Categories from FRAC Code List ©. FRAC code uses numbers and letters to distinguish fungicide groups according to their cross resistance behaviour.

A new method of detecting rhynchosporium using Polymerase Chain Reaction technology (PCR) has been developed at Rothamsted Research (Fountaine 2005). This tool allows us to identify the presence of rhynchosporium DNA in crops before symptoms are seen. As this diagnostic technology becomes more widely used, the challenge to advisers is to interpret results in situations where rhynchosporium is detected in a crop but there are no visual symptoms.

This research will address this issue and assist growers to determine if the presence of rhynchosporium DNA at the time of treatment increases the risk of future disease compared to a crop where no rhynchosporium DNA can be detected.

New PCR methods also provide a useful tool for research. By collecting rhynchosporium DNA from untreated and treated trials from a range of sites, the material can be used as part of other complementary research programmes to understand the population dynamics and mutations associated with fungicide resistance.

Materials and Methods

Field trials

Five field trials were sown in each of three years in Scotland and Northern Ireland, providing a total of six winter barley trials and nine spring barley trials. The varieties used were susceptible to rhynchosporium to ensure the best possible chance of rhynchosporium developing. In Scotland, the winter barley variety used was Haka and the spring barley variety Braemar. In Northern Ireland, the spring barley variety was Annabell. A summary of the trial locations is given in Table 2.

Table 2 Location of field trials 2005-2007

Trial number	Crop	Variety	Site	Address	Grid reference
0501	Winter barley	Haka	N Scotland	Tipperty Farms, Tarsets, Ellon	NJ 965 275
0502	Winter barley	Haka	Central Scotland	Ploughlands, Mertoun Farms, Kelso	NT 627 308
0503	Spring Barley	Braemar	N Scotland	Tipperty Farms, Tarsets, Ellon	NJ 967 282
0504	Spring Barley	Braemar	Central Scotland	Balgonie, Glenrothes, Fife	NO 316 000
0505	Spring Barley	Annabell	N Ireland	Crossnacreevy, Co.Down, Northern Ireland	J 393 695
0601	Winter barley	Haka	N Scotland	Tipperty Farms, Tarsets, Ellon	NJ 963 275
0602	Winter barley	Haka	Central Scotland	Ploughlands, Mertoun Farms, Kelso	NT 623 312
0603	Spring Barley	Braemar	N Scotland	Tipperty Farms, Tarsets, Ellon	NJ 977 241
0604	Spring Barley	Braemar	Central Scotland	Skeddoway, Thornton, Fife	NT 254 982
0605	Spring Barley	Annabell	N Ireland	Crossnacreevy, Co.Down, Northern Ireland	J 393 695
0701	Winter barley	Haka	N Scotland	Tipperty Farms, Tarsets, Ellon	NJ 958 274
0702	Winter barley	Haka	Central Scotland	Ploughlands, Mertoun Farms, Kelso	NT 643 332
0703	Spring Barley	Braemar	N Scotland	Tipperty Farms, Tarsets, Ellon	NJ 984 284
0704	Spring Barley	Braemar	Central Scotland	Skeddoway, Thornton, Fife	NT 251 981
0705	Spring Barley	Annabell	N Ireland	Crossnacreevy, Co.Down, Northern Ireland	J 393 695

Fungicide treatments were applied using a knapsack sprayer with medium nozzles in 200 litres water/ha. Routine treatments of fertiliser, herbicides and insecticides were applied over the whole trial site. Table 3 provides details of the fungicide treatment spray dates in all trials.

Table 3 Fungicide spray dates

Trial number	Early fungicide		Late fungicide		Interval between sprays
	Date	Growth stage	Date	Growth stage	Days
0501	22 April 05	GS31-32	7 June 05	GS59	46
0502	21 April 05	GS31-32	6 June 05	GS59	45
0503	7 June 05	GS31-32	7 July 05	GS59	31
0504	8 June 05	GS31-32	5 July 05	GS59	28
0505	20 Jun 05	GS30	8 July 05	GS55	18
0601	2 May 06	GS31-32	8 June 06	GS59	37
0602	28 Apr 06	GS31-32	5 June 06	GS59	38
0603	8 Jun 06	GS31-32	10 July 06	GS59	33
0604	25 May 06	GS31-32	7 July 06	GS59	43
0605	20 Jun 06	GS30	8 July 06	GS55	18
0701	24 Apr 07	GS31-32	24 May 07	GS59	31
0702	17 Apr 07	GS31-32	15 May 07	GS59	31
0703	24 May 07	GS25-30	9 July 07	GS59	46
0704	14 May 07	GS25-30	25 June 07	GS59	48
0705	6 June 07	GS30	18 July 07	GS59	41

The fungicide treatments applied are listed in Table 4 as the trade names of the fungicides, dose rates applied and the total equivalent dose where one is the equivalent of one full recommended dose. The code shows the treatment abbreviations which have been used in the results and discussion sections.

The interval between treatments varied between seasons, with an average interval of 28 days in the winter sown crops and 34 days in the spring sown crops.

Table 4 Fungicide treatments, trade names

Fungicide treatment					
Code	**Fungicide 1**	**Fungicide 2**	**Fungicide 3**	**Dose**	**£/ha**
Nil	Nil	-	-	0	4.0
P	Proline 0.4 l/ha	-	-	0.5	19.2
C	Corbel 0.5 l/ha	-	-	0.5	15.0
V	Vivid 0.5 l/ha	-	-	0.5	16.5
U	Unix 0.4 kg/ha	-	-	0.5	12.3
B	Bravo 1.0 l/ha	-	-	0.5	8.0
PC	Proline 0.4 l/ha	Corbel 0.5 l/ha	-	1.0	30.2
PV	Proline 0.4 l/ha	Vivid 0.5 l/ha	-	1.0	31.7
PU	Proline 0.4 l/ha	Unix 0.4 kg/ha	-	1.0	27.5
PB	Proline 0.4 l/ha	Bravo 1.0 l/ha	-	1.0	23.2
CV	Corbel 0.5 l/ha	Vivid 0.5 l/ha	-	1.0	27.5
CU	Corbel 0.5 l/ha	Unix 0.4 kg/ha	-	1.0	23.3
CB	Corbel 0.5 l/ha	Bravo 1.0 l/ha	-	1.0	19.0
VU	Vivid 0.5 l/ha	Unix 0.4 kg/ha	-	1.0	24.8
VB	Vivid 0.5 l/ha	Bravo 1.0 l/ha	-	1.0	20.5
UB	Unix 0.4 kg/ha	Bravo 1.0 l/ha	-	1.0	16.3
PCV	Proline 0.4 l/ha	Corbel 0.5 l/ha	Vivid 0.5 l/ha	1.5	42.7
PCU	Proline 0.4 l/ha	Corbel 0.5 l/ha	Unix 0.4 kg/ha	1.5	38.5
PCB	Proline 0.4 l/ha	Corbel 0.5 l/ha	Bravo 1.0 l/ha	1.5	34.2
PVU	Proline 0.4 l/ha	Vivid 0.5 l/ha	Unix 0.4 kg/ha	1.5	40.0
PVB	Proline 0.4 l/ha	Vivid 0.5 l/ha	Bravo 1.0 l/ha	1.5	35.7
PUB	Proline 0.4 l/ha	Unix 0.4 kg/ha	Bravo 1.0 l/ha	1.5	31.5
VCU	Vivid 0.5 l/ha	Corbel 0.5 l/ha	Unix 0.4 kg/ha	1.5	35.8
VCB	Vivid 0.5 l/ha	Corbel 0.5 l/ha	Bravo 1.0 l/ha	1.5	31.5
VUB	Vivid 0.5 l/ha	Unix 0.4 kg/ha	Bravo 1.0 l/ha	1.5	28.8
UCB	Unix 0.4 kg/ha	Corbel 0.5 l/ha	Bravo 1.0 l/ha	1.5	27.3
Overspray					
Bravo 1.0 l/ha					

All plots, including “Nil” received an overspray of Bravo at 1.0 l/ha at the ear emerging stage after all samples had been taken. More details of the active ingredients are provided in Table 5.

Table 5 Fungicide treatment active ingredients

		Active ingredients g/ha at GS25-32		
No.	**Code**	**Active ingredient 1**	**Active ingredient 2**	**Active ingredient 3**
1	Nil	-	-	-
2	P	prothioconazole 100	-	-
3	C	fenpropimorph 375	-	-
4	V	pyraclostrobin 125	-	-
5	U	cyprodinil 300	-	-
6	B	chlorothalonil 500	-	-
7	PC	prothioconazole 100	fenpropimorph 375	-
8	PV	prothioconazole 100	pyraclostrobin 125	-
9	PU	prothioconazole 100	cyprodinil 300	-
10	PB	prothioconazole 100	chlorothalonil 500	-
11	CV	fenpropimorph 375	pyraclostrobin 125	-
12	CU	fenpropimorph 375	cyprodinil 300	-
13	CB	fenpropimorph 375	chlorothalonil 500	-
14	VU	pyraclostrobin 125	cyprodinil 300	-
15	VB	pyraclostrobin 125	chlorothalonil 500	-
16	UB	cyprodinil 300	chlorothalonil 500	-
17	PCV	prothioconazole 100	fenpropimorph 375	pyraclostrobin 125
18	PCU	prothioconazole 100	fenpropimorph 375	cyprodinil 300
19	PCB	prothioconazole 100	fenpropimorph 375	chlorothalonil 500
20	PVU	prothioconazole 100	pyraclostrobin 125	cyprodinil 300
21	PVB	prothioconazole 100	pyraclostrobin 125	chlorothalonil 500
22	PUB	prothioconazole 100	cyprodinil 300	chlorothalonil 500
23	VCU	pyraclostrobin 125	fenpropimorph 375	cyprodinil 300
24	VCB	pyraclostrobin 125	fenpropimorph 375	chlorothalonil 500
25	VUB	pyraclostrobin 125	cyprodinil 300	chlorothalonil 500
26	UCB	cyprodinil 300	fenpropimorph 375	chlorothalonil 500
	Overspray to all treatments at ear emergence			
	chlorothalonil 500 g			

Disease assessments in field

Initially plots were assessed on a plot basis (% infection). At the first fungicide treatment, the uppermost fully emerged leaf was tagged using a small parcel tag on approximately 5 plants in plot 1. This enabled assessors to determine which leaf was emerging at the time of treatment and link leaf layers to the flag leaf. When the final leaf had emerged (post GS39), it was possible to relate the leaf layers to the flag leaf.

Rhynchosporium was assessed at the time of fungicide treatment. Trials were assessed at approximately two week intervals after treatment. Since disease assessments would not be done on all sites at the same time, assessments were classified on the basis of 14, 28 and 42 days after the first treatment. At the time of the overspray (GS55-59), an assessment was done on all trials.

Protectant activity was defined as prevention of the development of disease on the upper leaves which emerged after the first fungicide treatment (flag leaf and leaf 2). Eradicant activity was defined as prevention of development of disease on the lower leaves which had emerged or were emerging at the time of the treatment (usually leaves 3-5) after treatment.

Harvest assessments and economic response to treatment

At harvest, individual plot yields and dry matter were recorded. Post harvest the specific weight was determined.

The economic response to the treatments was calculated from the grain yield less the cost of the fungicides to give margin over fungicide prices. Three different price levels were chosen for:

Grain values for margin over fungicide price	**£/tonne**
Low	75
Medium	125
High	175

DNA extraction and quantification

The objective of this research was to apply quantitative real-time PCR assays to detect and quantify infection levels of *R. secalis*. Plant tissues from untreated and fungicide treated winter and spring barley crops were sampled at several time points during the growing season and data used to understand how fungicides can control epidemics. DNA was extracted from all plant tissues (10 roots, shoots or leaves per sample) and fungal material (calibration curve samples) using the method of Fraaije et al. (1999) except that the DNA extraction buffer was amended with 5 mM 1,10-phenanthroline monohydrate and 2 % (wt/vol) polyvinylpyrrolidone K30 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (Zhang et al., 2000). The DNA was quantified using the fluorescent dye thiazole orange (Sigma-Aldrich) as described previously (Fraaije et al., 2005)).

Quantitative real-time PCR using LNA probes to detect *Rhynchosporium secalis*

The quantitative PCR assay used for this study was developed by Fountaine *et al*. (2007). This PCR assay targets the mitochondrial cytochrome *b* gene and a fluorescence labelled Locked Nucleic Acid (LNA) probe is used to quantify the accumulation of PCR products in time. To perform the assay, each well contained 5 µl of template DNA and 15 µl of reaction mix. Reaction mix consisted of 10 µl of PCR master mix (JumpStart^{tm} *Taq* ReadyMix^{tm}, 20 mM Tris HCl (pH 8.3), 100 mM KCl, 3 mM $MgCl_2$, 0.002 % gelatine, 0.4 mM of each dNTP, Stabilizers, 0.06 unit μl^{-1} *Taq* DNA polymerase, JumpStart *Taq* antibody) (Sigma-Aldrich, Saint Louis, Missouri, USA), 400 nM forward primer Rsrtpcr1f, 400 nM reverse primer Rsrtpcr1r, 100 nM LNA fluorogenic probe Rsrtpcr1p (see below for nucleotide sequences), 0.1 µl 50 x ROX reference dye (Sigma-Aldrich) and sterile distilled water. Amplification of the 125 bp PCR product was carried out in a Stratagene Mx3000P real-time PCR machine (Stratagene, La Jolla, California, USA) under the following conditions: 1 cycle at 94 ^oC for 10 mins, followed by 50 cycles at 95 ^oC for 15 s, 60 ^oC for 1 min. The increase of fluorescence from the probe was recorded at 60 ^oC.

For all real-time PCR assays, samples were run in duplicate. For each sample, the threshold cycle (cycle at which increase of fluorescence exceeded the background (Ct)) was determined. Samples detected earlier than control samples (50 ng DNA extracted from 'clean' barley leaves sampled from plants grown in a glasshouse) were regarded as positive. Plotting known amounts of target DNA against Ct values generated standard curves. Calibration curves were generated by spiking barley leaf DNA samples (50 ng of DNA) with different amounts of genomic *R. secalis* DNA,

ranging from 0.0256 pg to 50 ng. Standard curve samples were always run simultaneously with test samples in each real-time PCR experiment.

Oligonucleotides used for the quantitative real-time PCR measurements according Fountaine *et al*. (2007)

Oligonucleotide name	Oligonucleotide type	Sequences (5`-3`) and labelling
Rsrtpcr1f	Forward primer	ATGTGCTTCCTTATGGACAGATGT
Rsrtpcr1r	Reverse primer	ATTATTAACAGAAAAACCCCCTCAGAT
Rsrtpcr1p	LNA probe	FAM-TATG*AG*CTGCC*AC*AGT-BHQ-1[1]

[1] Probe labelled with 6-carboxy fluorescein (FAM) and black hole quencher BHQ-1. Asterisks in front of nucleotides indicate which bases have 2'-O, 4'-C methylene linkages.

In order to analyse and compare data for all PCR runs, we used a detection threshold of 1.0 pg of pathogen DNA per sample. Samples below this threshold were regarded as zero in the analysis.

Analysis of trial data

All the data were analysed using randomised block analyses of variance. It was found advantageous to use the treatment formula, Number/Treatment, in the analyses, where Number is the number of component fungicides in the Treatment level. This analysis allows the Control to be tested against the single fungicides, the two-way and the three-way fungicide mixtures. The tests of significance for "Treatment" are consequently tests of the evidence for differences within these groups. The treatment means were subsequently used in the over-trial analyses. Only the over-trial analyses are reported in this document.

Trial classification

The 15 trials were classified and grouped as a range of factors for analysis (see Table 6a and 6b). The most obvious classifications were year, variety and sowing date (winter or spring barley). Once a preliminary analysis of the trials had been done, further classifications were possible on the basis of disease severity, fungicide resistance, *R secalis* DNA levels and spring rain.

The purpose of adding a categorisation factor into the analysis is to test the hypothesis that some treatment levels show different responses in some categories of trial.

For example, it is of interest to test the hypothesis that the response to a given treatment is different for spring and winter crops.

A more speculative hypothesis might be that crops showing higher *R secalis* DNA in the spring are those that benefit from treatment most.

It is important that the Trial categorisations done prior to treatment are not confused with categorisations done post treatment. The latter could never be used to identify when treatments would be most efficacious. The post treatment categories are essentially responses to previous categorisations.

Keys for Table 6a and 6b

Label	Category	Code
Sowing		
Winter		1
Spring		2
Variety		
Annabell		1
Braemar		2
Haka		3
Region		
Northern Ireland		1
Scotland south		2
Scotland north		3
R secalis DNA	**pg DNA**	
Nil	<1	1
Low	1-50	2
Mod	51-500	3
High	501-5000	4
Vhigh	5001-50000	5
Visual Disease		
Nil	0	1
Low	0.1-5	2
Mod	5.1-10	3
High	>10.1	4
Prothioconazole (P) resistance		
x	Limited data	
Low	0.001-3.33	
Mod	3.34-10.0	
High	>10.0-40.0	
Spring Rain		
Low	Met office lower than average March, April	1
High	Met office higher than average March, April	2

Table 6a Classification of Trials for analysis

						Visual symptoms April/May	Early R secalis DNA	Early R secalis DNADNA	Early R secalis DNA
Trial	**Sowing**	**Variety**	**Year**	**Site**	**Region**	**Early disease**	**Root**	**ShootLeaf**	**Leaves**
0501	1	3	1	1	3	2	1	2	1
0502	1	3	1	2	2	2	3	3	1
0503	2	2	1	3	3	1	1	1	2
0504	2	2	1	4	2	1	2	2	1
0505	2	1	1	5	1	1	1	2	1
0601	1	3	2	1	3	2	2	3	2
0602	1	3	2	2	2	2	4	3	2
0603	2	2	2	3	3	1	2	1	1
0604	2	2	2	4	2	1	1	1	1
0605	2	1	2	5	1	1	3	1	1
0701	1	3	3	1	3	1	4	3	2
0702	1	3	3	2	2	2	4	3	2
0703	2	2	3	3	3	1	3	1	1
0704	2	2	3	4	2	1	2	1	1
0705	2	1	3	5	1	2	3	3	2

Table 6b Classification of Trials for analysis

						Late DNA	Visual Symptoms June/July			prothioconazole resistance
Trial	**Sowing**	**Variety**	**Year**	**Site**	**Region**	**Leaf 2**	**Late disease**	**Spring rain**	**Summer rain**	**Prothioconazole resistance**
0501	1	3	1	1	3	1	2	2	1	2
0502	1	3	1	2	2	1	2	2	1	2
0503	2	2	1	3	3	1	2	2	1	
0504	2	2	1	4	2	2	2	2	1	
0505	2	1	1	5	1	2	2	2	1	1
0601	1	3	2	1	3	1	2	2	1	3
0602	1	3	2	2	2	1	2	2	1	2
0603	2	2	2	3	3	1	1	2	1	
0604	2	2	2	4	2	1	1	2	1	
0605	2	1	2	5	1	1	1	2	1	
0701	1	3	3	1	3	2	1	1	2	3
0702	1	3	3	2	2	1	1	1	2	3
0703	2	2	3	3	3	2	1	1	2	
0704	2	2	3	4	2	1	1	1	2	
0705	2	1	3	5	1	2	1	1	2	1

Over-trial analysis

Each of the fifteen trials in this study had the same treatment design and consequently it was found possible to use analysis of variance for the over-trial analysis of the Trial x Treatment means. There are many advantages in using this statistical methodology.

The simplest analysis regarded Trial as a “block” factor and Treatment (with 26 levels” as the “treatment” factor.

Using the trial categories of Table 6a and 6b, it was possible to test these factors in the Trial strata and also their interaction with the Treatment factor. Thus:

Block Trial

Treatment Category*Treatment

The next stage of development of the analysis was to use the Number/Treatment structure of the Treatment factor. In this context, Number is the number of fungicides in a Treatment level. This allows the evidence for overall differences between groups of treatment levels with the same number of fungicides to be assessed. In this context Treatment tests the evidence for differences between treatment levels with the same number of fungicides. The analysis formulae used were thus:

Block Trial
Treatment Category*(Number/Treatment)

This formulation of the analysis leads to 5 tests of significance and makes full use of the structure of the data.

Categorisation of the trials allows the hypothesis that the Treatments have different effects in different categories, e.g. Spring or Winter, of trial to be tested.

The %disease data and %green leaf data have been transformed for over trial analysis using the empirical logit transformation.

Transformed Value = log (x + 0.5) / log (100 + 0.5 – x) where x is a % figure.

By adding 0.5 to the numerator and denominator, 0% and 100% can be adequately dealt with.

For DNA data the transformation used was log (x + 0.5) where x is the quantity of DNA measured in pg.

All analysis has been reported on the transformed scale. Results on this scale should be used for statistical testing. For transformed data, the back-transform of the Treatment x Category tables has been provided to aid interpretation.

Results

The codes for treatments are described in Tables 4 and 5 in the Materials and Methods. The product codes are repeated here to assist reading the results tables. Tables show the results in treatment order. Where results are in transformed data format, (disease, green leaf and DNA levels) a table of actual values is also presented. The figures represent non transformed data and have been ranked in order of treatment activity.

In the analysis the following definitions have been used:

Sowing = winter or spring sown crop.
Number = Number of individual fungicides used in the mixture
Treatment = individual fungicide mixture.
The **Av SED** is the average of the standard errors for all pair wise comparisons within a Table.

Fungicide treatment					
Code	**Fungicide 1**	**Fungicide 2**	**Fungicide 3**	**Number**	**£/ha**
Nil	Nil	-	-	0	4.0
P	Proline 0.4 l/ha	-	-	1	19.2
C	Corbel 0.5 l/ha	-	-	1	15.0
V	Vivid 0.5 l/ha	-	-	1	16.5
U	Unix 0.4 kg/ha	-	-	1	12.3
B	Bravo 1.0 l/ha	-	-	1	8.0
PC	Proline 0.4 l/ha	Corbel 0.5 l/ha	-	2	30.2
PV	Proline 0.4 l/ha	Vivid 0.5 l/ha	-	2	31.7
PU	Proline 0.4 l/ha	Unix 0.4 kg/ha	-	2	27.5
PB	Proline 0.4 l/ha	Bravo 1.0 l/ha	-	2	23.2
CV	Corbel 0.5 l/ha	Vivid 0.5 l/ha	-	2	27.5
CU	Corbel 0.5 l/ha	Unix 0.4 kg/ha	-	2	23.3
CB	Corbel 0.5 l/ha	Bravo 1.0 l/ha	-	2	19.0
VU	Vivid 0.5 l/ha	Unix 0.4 kg/ha	-	2	24.8
VB	Vivid 0.5 l/ha	Bravo 1.0 l/ha	-	2	20.5
UB	Unix 0.4 kg/ha	Bravo 1.0 l/ha	-	2	16.3
PCV	Proline 0.4 l/ha	Corbel 0.5 l/ha	Vivid 0.5 l/ha	3	42.7
PCU	Proline 0.4 l/ha	Corbel 0.5 l/ha	Unix 0.4 kg/ha	3	38.5
PCB	Proline 0.4 l/ha	Corbel 0.5 l/ha	Bravo 1.0 l/ha	3	34.2
PVU	Proline 0.4 l/ha	Vivid 0.5 l/ha	Unix 0.4 kg/ha	3	40.0
PVB	Proline 0.4 l/ha	Vivid 0.5 l/ha	Bravo 1.0 l/ha	3	35.7
PUB	Proline 0.4 l/ha	Unix 0.4 kg/ha	Bravo 1.0 l/ha	3	31.5
VCU	Vivid 0.5 l/ha	Corbel 0.5 l/ha	Unix 0.4 kg/ha	3	35.8
VCB	Vivid 0.5 l/ha	Corbel 0.5 l/ha	Bravo 1.0 l/ha	3	31.5
VUB	Vivid 0.5 l/ha	Unix 0.4 kg/ha	Bravo 1.0 l/ha	3	28.8
UCB	Unix 0.4 kg/ha	Corbel 0.5 l/ha	Bravo 1.0 l/ha	3	27.3

Overspray
Bravo 1.0 l/ha

Crop yields

Winter sown crops achieved higher yields than spring sown crops (Table 7). Increasing fungicide dose also increased yield.

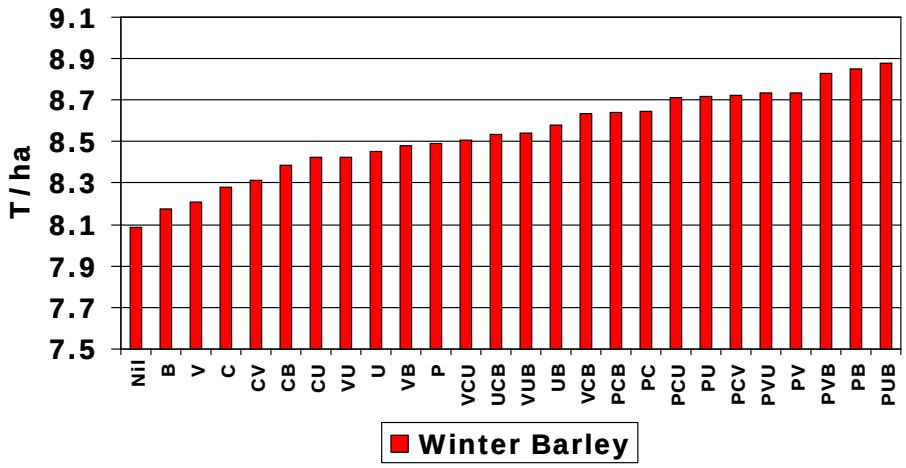

Figure 1 Winter barley yields

Figure 1 shows the yields for winter barley in ascending order. Best yielding programmes included prothioconazole and chlorothalonil components. Single fungicides and fungicides including fenpropimorph were amongst the lower yielding mixtures.

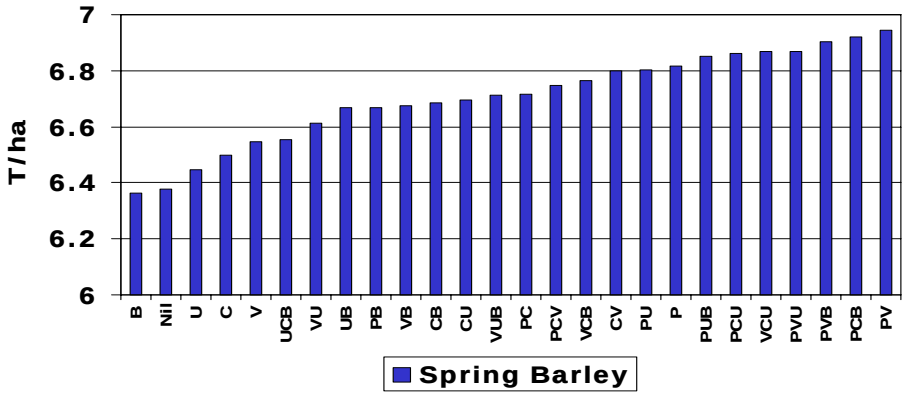

Figure 2 Spring barley crop yields

Yield responses were lower in the spring barley. Prothioconazole was the key component to the higher yielding mixtures and prothioconazole + pyraclostrobin achieved the best yield.

Table 7 Crop yields (t/ha) from 2005-2007 trials

Sowing	Winter		Spring	
	8.54		6.71	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	7.06	7.25	7.46	7.55
Winter	8.09	8.32	8.56	8.67
Spring	6.38	6.53	6.73	6.80

	Weighted Mean	Winter	Spring
Nil	7.06	8.09	6.38
P	7.49	8.49	6.82
C	7.21	8.28	6.50
V	7.21	8.21	6.54
U	7.25	8.45	6.44
B	7.09	8.17	6.36
PC	7.49	8.65	6.72
PV	7.66	8.74	6.94
PU	7.57	8.72	6.80
PB	7.54	8.85	6.67
CV	7.41	8.32	6.80
CU	7.39	8.42	6.69
CB	7.37	8.39	6.68
VU	7.34	8.43	6.61
VB	7.40	8.48	6.68
UB	7.43	8.58	6.67
PCV	7.54	8.72	6.75
PCU	7.60	8.71	6.86
PCB	7.61	8.64	6.92
PVU	7.61	8.73	6.87
PVB	7.67	8.83	6.90
PUB	7.66	8.88	6.85
VCU	7.52	8.51	6.87
VCB	7.51	8.64	6.76
VUB	7.44	8.54	6.71
UCB	7.35	8.54	6.55

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	0.516	0.078	0.529	0.106	0.537
Sig.	0.004	<0.001	Ns	0.001	Ns

Specific weights

Specific weights were all within market requirements and fungicides had little impact (Table 8). This suggests all treatments achieved grain of acceptable quality for specific weight.

Table 8 Specific weights (kg/hl) from 2005-2007 trials

Sowing	Winter		Spring	
	66.69		64.84	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	65.52	65.58	65.75	65.71
Winter	66.13	66.52	66.84	66.69
Spring	64.99	64.78	64.81	64.88

	Weighted Mean	Winter	Spring
Nil	65.52	66.13	64.99
P	65.52	66.42	64.75
C	65.62	66.41	64.95
V	65.84	66.83	94.99
U	65.36	66.40	64.46
B	65.57	66.54	64.74
PC	65.57	66.67	64.63
PV	65.98	66.81	65.27
PU	65.93	67.06	64.97
PB	66.08	67.46	64.90
CV	65.64	66.88	64.59
CU	65.60	66.72	64.63
CB	65.68	66.44	65.03
VU	65.58	66.85	64.50
VB	65.89	66.87	65.04
UB	65.50	66.65	64.52
PCV	65.70	66.89	64.69
PCU	65.79	66.84	64.89
PCB	65.95	66.84	65.18
PVU	65.73	66.89	64.74
PVB	65.84	66.79	65.04
PUB	65.70	66.11	65.35
VCU	65.57	66.60	64.68
VCB	65.47	66.50	64.58
VUB	65.62	66.70	64.69
UCB	65.76	66.72	64.94

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	2.798	0.184	2.810	0.248	2.819
Sig.	Ns	Ns	0.06	Ns	Ns

Margin over fungicide costs (£/ha)

At a high grain price (£175/t), higher margins were achieved in the winter barley as a consequence of the higher yield response to fungicide (Table 9). Increasing dose also led to an increase in margin, with three-way mixtures achieving the best.

At £125/t a similar pattern was seen with increasing the number of components in the mixture increasing the margin (Table 10).

At £75/t the two-way mixtures achieved the best margins (Table 11).

Table 9 Margins High – grain price £175/t, from 2005-2007 trials

Sowing	Winter	Spring
	1468	1148

	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	1232	1254	1281	1287
Winter	1412	1442	1473	1483
Spring	1112	1129	1153	1156

	Weighted Mean	Winter	Spring
Nil	1232	1412	1112
P	1291	1467	1174
C	1247	1434	1122
V	1245	1420	1129
U	1256	1467	1116
B	1232	1422	1106
PC	1280	1483	1145
PV	1309	1497	1184
PU	1297	1498	1163
PB	1296	1526	1144
CV	1268	1428	1162
CU	1269	1451	1148
CB	1270	1449	1151
VU	1259	1450	1132
VB	1274	1464	1148
UB	1284	1485	1150
PCV	1276	1483	1138
PCU	1292	1486	1162
PCB	1297	1478	1177
PVU	1292	1488	1162
PVB	1307	1509	1172
PUB	1309	1522	1167
VCU	1281	1453	1166
VCB	1283	1480	1152
VUB	1274	1466	1146
UCB	1258	1467	1119

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	90.3	13.7	92.6	18.5	94.0
Sig.	0.004	<.001	Ns	0.03	Ns

Table 10 Margins - Medium grain price £125/t, from 2005-2007 trials

Sowing	Winter		Spring	
	1042		813	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	879	892	908	909
Winter	1007	1026	1045	1050
Spring	793	802	816	816

	Weighted Mean	Winter	Spring
Nil	879	1007	793
P	917	1042	833
C	886	1020	797
V	885	1009	802
U	894	1044	793
B	878	1014	788
PC	906	1051	809
PV	926	1060	836
PU	919	1062	823
PB	919	1083	810
CV	898	1012	822
CU	900	1030	813
CB	902	1030	817
VU	892	1028	802
VB	904	1040	814
UB	913	1056	817
PCV	900	1047	801
PCU	912	1050	819
PCB	917	1046	831
PVU	912	1052	819
PVB	924	1068	827
PUB	926	1078	825
VCU	905	1027	823
VCB	908	1048	814
VUB	902	1039	810
UCB	891	1040	792

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	64.5	9.8	66.2	13.2	67.2
Sig.	0.004	<.001	Ns	0.05	Ns

Table 11 Margins - Low grain price £75/t, from 2005-2007 trials

	Number of Fungicides in Mixture			
	Nil	**1**	**2**	**3**
Weighted Mean	527	529	535	532
Winter	603	610	617	616
Spring	474	476	480	476

	Weighted Mean	**Winter**	**Spring**
Nil	527	603	474
P	542	618	492
C	526	606	472
V	524	599	474
U	531	622	471
B	524	605	469
PC	531	618	473
PV	542	623	489
PU	540	626	483
PB	542	641	477
CV	528	596	482
CU	531	609	479
CB	534	610	482
VU	526	607	471
VB	534	616	480
UB	541	627	484
PCV	523	611	464
PCU	532	615	476
PCB	536	614	485
PVU	531	615	475
PVB	539	626	482
PUB	543	634	482
VCU	529	602	479
VCB	532	616	476
VUB	529	612	475
UCB	524	613	464

	Sowing	**Number**	**Sowing x Number**	**Treatment**	**Sowing x Treatment**
Av. SED	38.7	5.9	39.7	7.9	40.3
Sig.	0.004	Ns	Ns	Ns	Ns

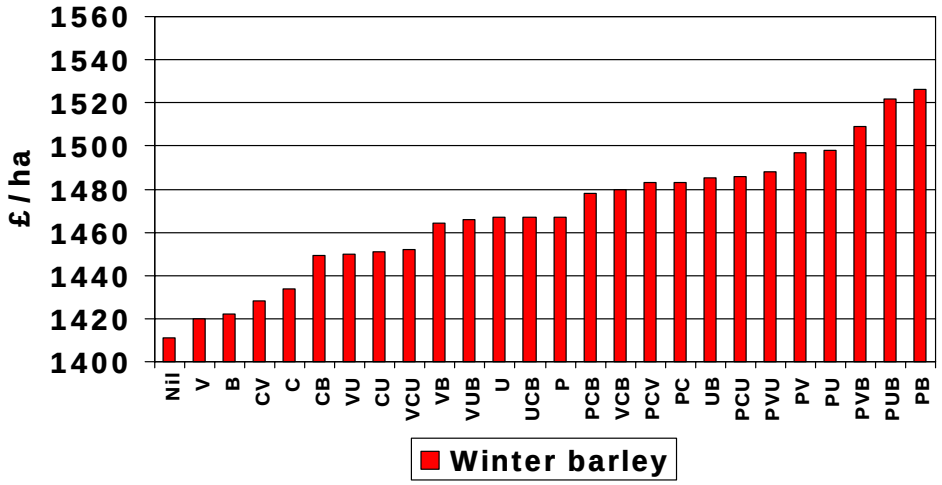

Figure 3 Margin over fungicide cost in winter barley at £175/t

Figure 3 shows the margins in ranked order for winter barley. The best margins were achieved from prothioconazole + chlorothalonil (PB) and prothioconazole + chlorothalonil + cyprodinil (PUB). The lower margin was achieved from the single fungicides pyraclostrobin (V) and chlorothalonil (B). Cyprodinil (U) and prothioconazole (P) however achieved a reasonable margin when used alone.

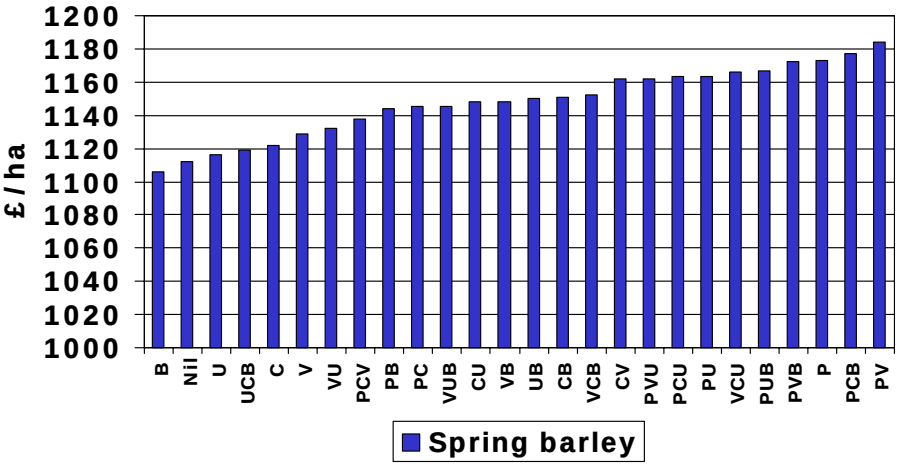

Figure 4 Margin over fungicide cost in spring barley at £175/t

Figure 4 shows the best margins in spring barley. Prothioconazole was a key component of the best treatments, but cyprodinil was less effective in spring barley compared to winter barley. Prothioconazole + pyraclostrobin (PV) and prothioconazole + chlorothalonil + fenpropimorph (PCB) achieved the best margins.

Protectant activity against rhynchosporium 28 days after treatment

Disease levels were low on the new growth 28 days after treatment (Tables 12, 13, Figure 5). Most treatments achieved good protection. Note chlorothalonil or prothioconazole used in mixtures gave the best protection.

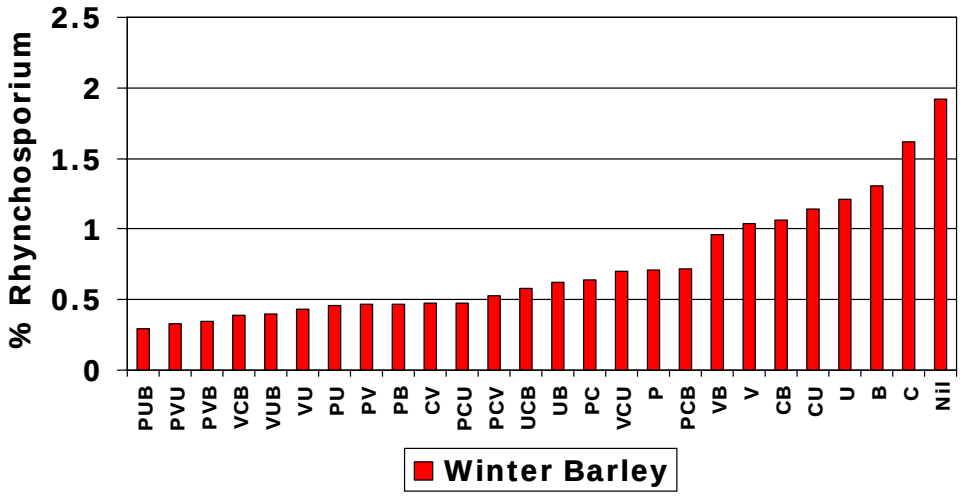

Figure 5 Protectant activity against rhynchosporium 28 days after treatment (winter barley)

Table 12 Protectant activity against rhynchosporium, 28 days after treatment (transformed percentages, empirical logit), from 2005-2007 trials

Sowing	Winter		Spring	
	-4.43		-4.21	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	-3.63	-4.04	-4.32	-5.52
Winter	-3.71	-4.10	-4.46	-4.63
Spring	-3.55	-3.98	-4.18	-4.42

	Weighted Mean	Winter	Spring
Nil	-3.63	-3.71	-3.55
P	-4.49	-4.41	-4.58
C	-3.65	-3.84	-3.46
V	-4.04	-4.17	-3.92
U	-4.06	-4.06	-4.48
B	-3.94	-4.00	-3.88
PC	-4.38	-4.47	-4.08
PV	-4.44	-4.63	-4.25
PU	-4.62	-4.65	-4.58
PB	-4.47	-4.64	-4.31
CV	-4.43	-4.62	-4.23
CU	-4.11	-4.10	-4.12
CB	-4.00	-4.16	-3.87
VU	-4.28	-4.67	-3.90
VB	-4.12	-4.22	-4.01
UB	-4.36	-4.48	-4.24
PCV	-4.57	-4.57	-4.56
PCU	-4.60	-4.62	-4.58
PCB	-4.37	-4.40	-4.33
PVU	-4.72	-4.78	-4.66
PVB	-4.66	-4.76	-4.55
PUB	-4.67	-4.83	-4.51
VCU	-4.47	-4.42	-4.52
VCB	-4.41	-4.71	-4.11
VUB	-4.43	-4.71	-4.16
UCB	-4.36	-4.53	-4.19

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	0.609	0.156	0.645	0.210	0.675
Sig.	Ns	<.001	Ns	0.02	Ns

Table 13 Protectant activity against rhynchosporium 28 days after treatment, % disease (back-transformation of Sowing x Treatment means from analysis)

	Winter	Spring
Nil	1.92	2.32
P	0.71	0.52
C	1.62	2.59
V	1.04	1.47
U	1.21	1.23
B	1.31	1.54
PC	0.64	0.87
PV	0.47	0.92
PU	0.46	0.52
PB	0.47	0.84
CV	0.48	0.94
CU	1.14	1.12
CB	1.06	1.63
VU	0.43	1.50
VB	0.96	1.29
UB	0.62	0.93
PCV	0.53	0.54
PCU	0.48	0.52
PCB	0.72	0.81
PVU	0.33	0.45
PVB	0.35	0.55
PUB	0.29	0.59
VCU	0.70	0.59
VCB	0.39	1.13
VUB	0.40	1.05
UCB	0.58	1.00

Protectant activity against rhynchosporium at time of overspray

Most two and three-way mixtures achieved good protection (Table 14, 15 and Figure 6). Prothioconazole alone was the best single fungicide.

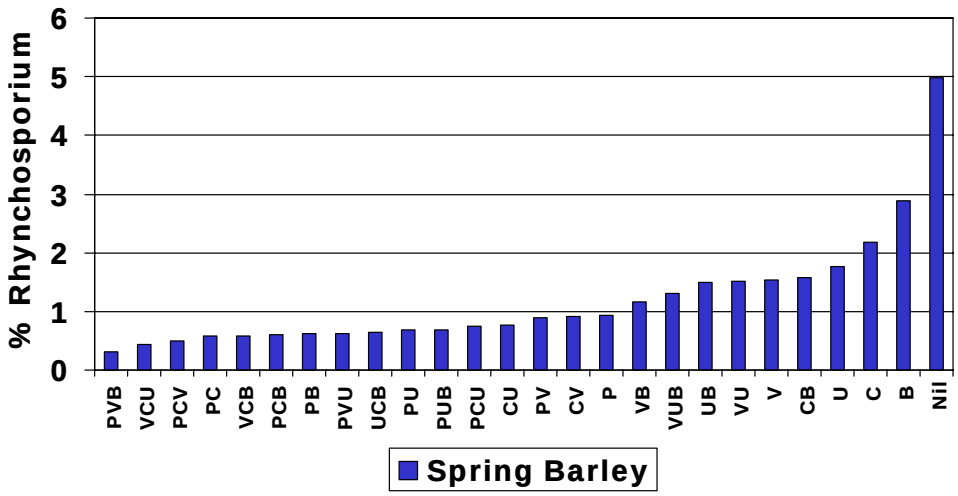

Figure 6 Protectant activity against rhynchosporium at time of overspray (spring barley)

Table 14 Protectant activity against rhynchosporium at time of overspray (GS55) (transformed percentages, empirical logit), from 2005-2007 trials

Sowing	Winter		Spring	
	-3.72		-4.18	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	-2.82	-3.61	-4.02	-4.25
Winter	-2.76	-3.38	-3.78	-3.94
Spring	-2.86	-3.77	-4.21	-4.49

	Weighted Mean	Winter	Spring
Nil	-2.82	-2.76	-2.86
P	-4.00	-3.68	-4.24
C	-3.49	-3.34	-3.60
V	-3.58	-3.17	-3.88
U	-3.55	-3.25	-3.77
B	-3.41	-3.48	-3.36
PC	-4.11	-3.55	-4.53
PV	-4.22	-4.16	-4.27
PU	-4.26	-4.03	-4.44
PB	-4.38	-4.23	-4.49
CV	-3.99	-3.63	-4.26
CU	-4.00	-3.51	-4.36
CB	-3.81	-3.73	-3.86
VU	-3.70	-3.45	-3.89
VB	-4.00	-3.88	-4.10
UB	-3.77	-3.59	-3.90
PCV	-4.38	-4.07	-4.61
PCU	-4.06	-3.64	-4.38
PCB	-4.36	-4.17	-4.51
PVU	-4.35	-4.17	-4.48
PVB	-4.44	-3.96	-4.80
PUB	-4.39	-4.32	-4.44
VCU	-4.32	-3.84	-4.68
VCB	-4.21	-3.78	-4.53
VUB	-3.90	-3.74	-4.01
UCB	-4.12	-3.65	-4.47

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	0.498	0.165	0.553	0.222	0.587
Sig.	Ns	<.001	Ns	0.02	Ns

Table 15 Protectant activity against rhynchosporium at time of overspray (GS55), % disease (back-transformation of Treatment x Sowing means from analysis)

	Winter	Spring
Nil	5.52	4.98
P	1.98	0.93
C	2.97	2.17
V	3.56	1.54
U	3.27	1.77
B	2.51	2.89
PC	2.33	0.58
PV	1.06	0.89
PU	1.26	0.68
PB	0.94	0.62
CV	2.11	0.91
CU	2.44	0.77
CB	1.86	1.58
VU	2.61	1.52
VB	1.54	1.16
UB	2.21	1.50
PCV	1.20	0.50
PCU	2.09	0.75
PCB	1.04	0.60
PVU	1.03	0.63
PVB	1.38	0.32
PUB	0.82	0.68
VCU	1.62	0.43
VCB	1.76	0.58
VUB	1.84	1.30
UCB	2.05	0.64

Rhynchosporium control as influenced by prothioconazole resistance

As part of the sensitivity testing in the next section, it was possible to define field sites as having low, moderate or high sensitive or resistance to prothioconazole (Tables 16, 17). A key question which these results address is the level of field activity from prothioconazole where isolates were less sensitive.

Table16 Protectant activity against rhynchosporium at time of overspray (GS55), categorised by resistance to prothioconazole (P) – (transformed percentages, empirical logit), from 2005-2007 trials

	Prothioconazole (P) resistance		
	Low	**Moderate**	**High**
Nil	-3.40	-2.24	-3.28
P	-3.74	-3.05	-4.31
C	-3.81	-2.46	-4.22
V	-3.88	-2.81	-3.54
U	-4.59	-2.72	-3.77
B	-3.22	-3.29	-3.68
PC	-4.86	-3.25	-3.84
PV	-3.42	-3.79	-4.52
PU	-3.92	-3.59	-4.48
PB	-4.25	-4.12	-4.34
CV	-3.92	-3.00	-4.27
CU	-4.23	-2.77	-4.25
CB	-3.99	-3.21	-4.25
VU	-3.73	-2.88	-4.02
VB	-4.24	-3.73	-4.03
UB	-3.96	-3.18	-4.01
PCV	-4.10	-4.00	-4.14
PCU	-4.34	-3.32	-3.95
PCB	-4.31	-3.87	-4.46
PVU	-4.10	-4.02	-4.33
PVB	-4.65	-3.65	-4.28
PUB	-4.67	-4.36	-4.28
VCU	-5.09	-3.54	-4.15
VCB	-4.13	-3.44	-4.12
VUB	-4.50	-3.34	-4.15
UCB	-4.34	-3.38	-3.92

	Resistance	**Number**	**Resistance x Number**	**Treatment**	**Resistance x Treatment**
Av. SED	0.811	0.199	0.896	0.734	0.943
Sig.	Ns	<.001	<.001	Ns	Ns

The results show that there were no significant differences between disease control at the sites categorised so prothioconazole still gives effective control of rhynchosporium where isolates were found to be less sensitive in laboratory tests.

Table 17 Protectant activity against rhynchosporium at time of overspray (GS55) (back-transformation of Resistance x Treatment means from analysis)

	Prothioconazole (P) resistance		
	Low	**Moderate**	**High**
Nil	2.8	9.2	3.2
P	1.8	4.1	0.8
C	1.7	7.5	1.0
V	1.5	5.2	2.4
U	0.5	5.7	1.8
B	3.4	3.1	2.0
PC	0.3	3.3	1.6
PV	2.7	1.7	0.6
PU	1.5	2.2	0.6
PB	0.9	1.1	0.8
CV	1.5	4.3	0.9
CU	0.9	5.5	0.9
CB	1.3	3.4	0.9
VU	1.9	4.9	1.3
VB	0.9	1.9	1.3
UB	1.4	3.5	1.3
PCV	1.1	1.3	1.1
PCU	0.8	3.0	1.4
PCB	0.8	1.6	0.6
PVU	1.1	1.3	0.8
PVB	0.5	2.1	0.9
PUB	0.4	0.8	0.9
VCU	0.1	2.4	1.1
VCB	1.1	2.7	1.1
VUB	0.6	3.0	1.1
UCB	0.8	2.8	1.5

Prothioconazole was the best single fungicide in these trials (Figure 7). It also gave best control in two and three-way mixtures. This suggests that even at sites where prothioconazole is less sensitive to rhynchosporium compared to the average, it remains the best component to achieve disease control.

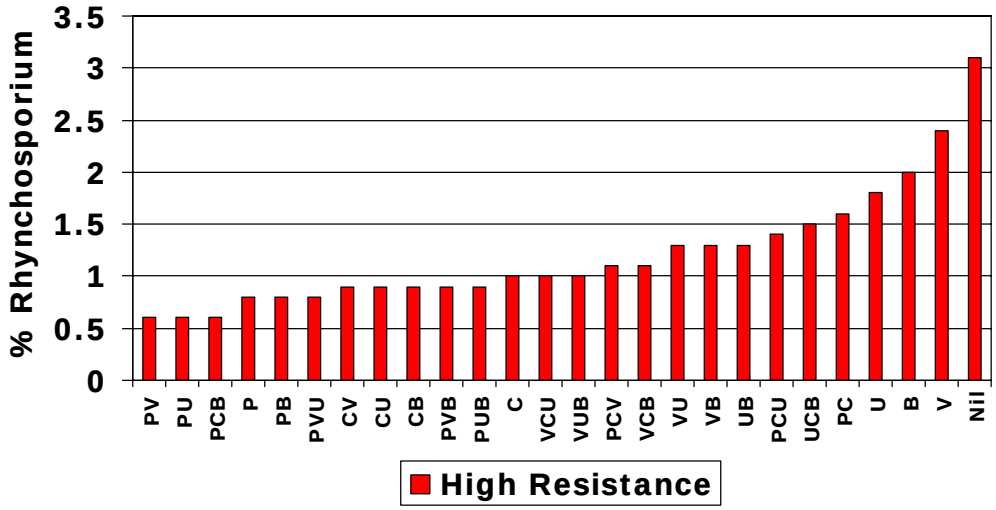

Figure 7 Disease control in prothioconazole resistant sites

Eradicant activity against rhynchosporium 28 days after treatment

Increasing dose gave best control. Disease levels were also higher in the winter barley crop (Tables 18, 19).

Pyraclostrobin was a key component of the mixture to achieve the best eradication (Figure 8). Pyraclostrobin used alone or in combination with chlorothalonil was however less effective. Fenpropimorph used alone gave poor control. This may be a reflection of poor persistence. It however performed well in mixture with pyraclostrobin.

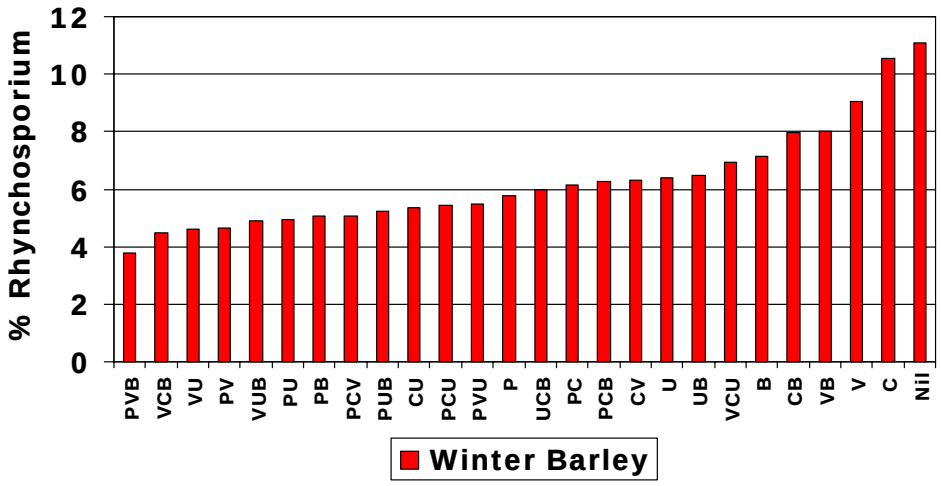

Figure 8 Eradicant activity against rhynchosporium 28 days after treatment (Winter barley)

Table 18 Eradicant activity against rhynchosporium 28 days after treatment (transformed percentages, empirical logit), from 2005-2007 trials.

Sowing	Winter		Spring	
	-2.66		-4.58	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	-2.85	-3.37	-3.56	-3.66
Winter	-2.04	-2.44	-2.70	-2.80
Spring	-3.82	-4.48	-4.60	-4.69

	Weighted Mean	Winter	Spring
Nil	-2.85	-2.04	-3.82
P	-3.54	-2.71	-4.52
C	-3.11	-2.10	-4.32
V	-3.36	-2.26	-4.69
U	-3.47	-2.62	-4.49
B	-3.35	-2.50	-4.37
PC	-3.72	-2.65	-5.00
PV	-3.78	-2.92	-4.82
PU	-3.71	-2.86	-4.73
PB	-3.71	-2.84	-4.76
CV	-3.59	-2.63	-4.74
CU	-3.58	-2.79	-4.54
CB	-3.27	-2.39	-4.32
VU	-3.46	-2.94	-4.09
VB	-3.36	-2.39	-4.52
UB	-3.46	-2.60	-4.49
PCV	-3.70	-2.84	-4.72
PCU	-3.80	-2.77	-5.04
PCB	-3.60	-2.64	-4.75
PVU	-3.68	-2.77	-4.76
PVB	-3.89	-3.12	-4.81
PUB	-3.78	-2.81	-4.94
VCU	-3.49	-2.54	-4.64
VCB	-3.56	-2.96	-4.28
VUB	-3.54	-2.87	-4.35
UCB	-3.58	-2.68	-4.65

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	0.394	0.150	0.448	0.202	0.484
Sig.	<.001	<.001	Ns	Ns	Ns

Table 19 Eradicant activity against rhynchosporium 28 days after treatment, % disease (back-transformation of Treatment x Sowing means from analysis)

	Winter	Spring
Nil	11.10	1.67
P	5.79	0.59
C	10.54	0.82
V	9.07	0.42
U	6.38	0.62
B	7.15	0.76
PC	6.17	0.18
PV	4.66	0.31
PU	4.96	0.38
PB	5.06	0.36
CV	6.30	0.38
CU	5.36	0.57
CB	7.98	0.82
VU	4.59	1.16
VB	8.00	0.59
UB	6.50	0.62
PCV	5.06	0.39
PCU	5.44	0.15
PCB	6.25	0.36
PVU	5.47	0.35
PVB	3.77	0.31
PUB	5.22	0.22
VCU	6.92	0.47
VCB	4.47	0.88
VUB	4.90	0.79
UCB	5.98	0.46

Rhynchosporium eradication in high disease pressure situations

Wet spring weather provides ideal conditions for rhynchosporium. This classification shows differences in disease control under this high disease pressure (Table 20).

Table 20 Eradicant activity against rhynchosporium 28 days after treatment - transformed percentages (empirical logit), from 2005-2007 trials classified by spring rainfall

Spring Rainfall	Dry		Wet	
	transformed	% disease	transformed	% disease
Nil	-3.62	2.1	-2.20	9.5
P	-3.86	1.6	-3.26	3.2
C	-3.88	1.6	-2.47	7.4
V	-4.22	1.0	-2.65	6.2
U	-4.13	1.1	-2.92	4.7
B	-4.03	1.3	-2.79	5.3
PC	-4.15	1.1	-3.35	2.9
PV	-3.88	1.5	-3.71	1.9
PU	-4.07	1.2	-3.41	2.7
PB	-4.00	1.3	-3.48	2.5
CV	-4.04	1.2	-3.21	3.4
CU	-4.12	1.1	-3.14	3.7
CB	-3.87	1.6	-2.76	5.5
VU	-3.91	1.5	-3.09	3.9
VB	-3.88	1.6	-2.92	4.6
UB	-4.05	1.2	-2.97	4.5
PCV	-3.94	1.4	-3.50	2.5
PCU	-4.22	1.0	-3.45	2.6
PCB	-4.07	1.2	-3.21	3.4
PVU	-3.98	1.3	-3.42	2.7
PVB	-4.09	1.2	-3.72	1.9
PUB	-4.22	1.0	-3.42	2.7
VCU	-3.84	1.6	-3.20	3.5
VCB	-3.76	1.8	-3.39	2.8
VUB	-3.86	1.6	-3.28	3.2
UCB	-4.13	1.1	-3.11	3.8

	Spring Rain	Number	Spring Rain x Number	Treatment	Spring Rain x Treatment
Av. SED	0.698	0.694	0.728	0.197	0.750
Sig.	Ns	<.001	Ns	Ns	Ns

Prothioconazole and pyraclostrobin were key components of the fungicide mixtures to eradicate disease in a wet spring (Figure 9).

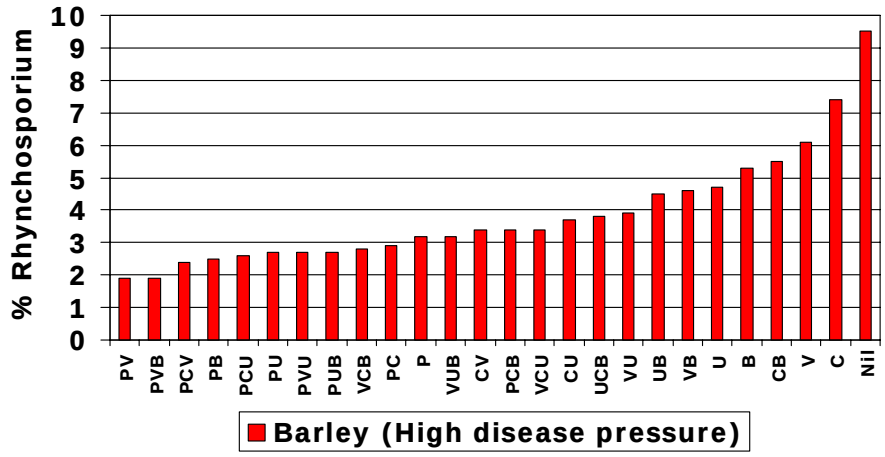

Figure 9 Eradicant activity against rhynchosporium in high disease pressure sites

A second high disease pressure scenario tested is where rhynchosporium was less sensitive (i.e. resistant) to prothioconazole. Results are shown in Table 21.

Table 21 Rhynchosporium eradication 28 days after treatment (transformed percentages, empirical logit), from 2005-2007 trials classified by prothioconazole resistance (P)

Resistance (P)	Moderate		High	
	transformed	% disease	transformed	% disease
Nil	-1.57	16.9	-2.52	7.0
P	-2.51	7.1	-2.92	4.7
C	-1.79	14.0	-2.41	7.8
V	-2.04	11.1	-2.47	7.4
U	-2.56	6.8	-2.67	6.0
B	-2.22	9.4	-2.79	5.4
PC	-2.58	6.6	-2.72	5.7
PV	-2.76	5.5	-3.08	3.9
PU	-2.77	5.4	-2.95	4.5
PB	-2.94	4.6	-2.74	5.6
CV	-2.48	7.3	-2.77	5.4
CU	-2.36	8.2	-3.21	3.4
CB	-2.16	10.0	-2.62	6.3
VU	-2.62	6.3	-3.25	3.3
VB	-2.34	8.4	-2.43	7.7
UB	-2.48	7.3	-2.72	5.8
PCV	-2.92	4.7	-2.77	5.5
PCU	-2.76	5.5	-2.78	5.4
PCB	-2.85	5.0	-2.42	7.7
PVU	-2.84	5.1	-2.70	5.9
PVB	-3.13	3.7	-3.11	3.8
PUB	-2.67	6.0	-2.96	4.5
VCU	-2.71	5.8	-2.36	8.2
VCB	-2.73	5.7	-3.19	3.5
VUB	-2.93	4.6	-2.82	5.2
UCB	-2.84	5.0	-2.52	7.1

	Resistance	Number	Resistance x Number	Treatment	Resistance x Treatment
Av. SED	0.610	0.175	0.751	0.236	0.789
Sig.	.07	0.002	<.001	Ns	Ns

Pyraclostrobin, cyprodinil, fenpropimorph and chlorothalonil were important components of the mixture in this high pressure situation in the prothioconazole-resistant sites (Figure 10). Prothioconazole however remained the best single fungicide.

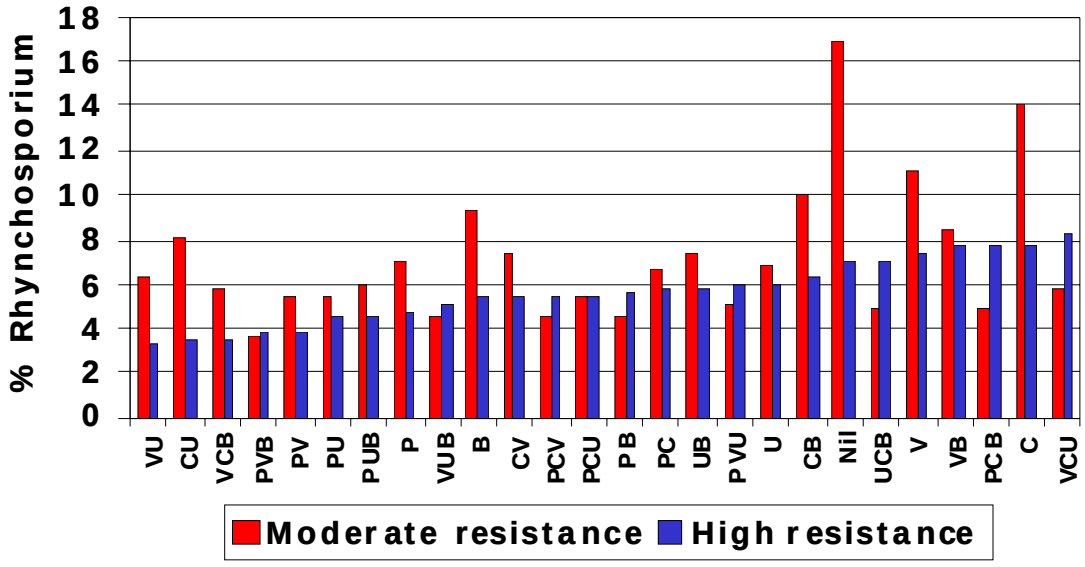

Figure 10 Rhynchosporium eradication in prothioconazole resistant sites

Use of diagnostics to forecast disease pressure

Table 22 shows disease levels 28 days after treatment where trials have been categorised by the initial amount of DNA detected.

Table 22 Eradicant activity against rhynchosporium 28 days after treatment (transformed percentages, empirical logit), from 2005-2007 trials classified by DNA on early leafs

Leaf DNA	Low		Moderate	
	Transformed	% Disease	Transformed	% Disease
Nil	-2.73	5.7	-2.99	4.3
P	-3.91	1.5	-3.08	3.9
C	-3.57	2.3	-2.56	6.8
V	-3.76	1.8	-2.88	4.8
U	-3.84	1.6	-3.02	4.2
B	-3.47	2.5	-3.21	3.4
PC	-4.20	1.0	-3.14	3.7
PV	-4.38	0.8	-3.15	3.7
PU	-4.25	0.9	-3.07	4.0
PB	-4.20	1.0	-3.13	3.7
CV	-3.95	1.4	-3.15	3.6
CU	-3.83	1.6	-3.29	3.1
CB	-3.72	1.9	-2.72	5.7
VU	-3.74	1.9	-3.14	3.7
VB	-3.66	2.0	-3.00	4.3
UB	-3.80	1.7	-3.04	4.1
PCV	-4.22	1.0	-3.06	4.0
PCU	-4.27	0.9	-3.24	3.3
PCB	-4.14	1.1	-2.95	4.5
PVU	-4.15	1.1	-3.11	3.8
PVB	-4.32	0.8	-3.38	2.8
PUB	-4.22	1.0	-3.26	3.2
VCU	-4.04	1.2	-2.83	5.1
VCB	-3.90	1.5	-3.15	3.7
VUB	-3.91	1.5	-3.11	3.8
UCB	-4.01	1.3	-3.06	4.0

	Leaf DNA	Total	Leaf DNA x Total	Treatment	Leaf DNA x Treatment
SED	0.695	0.148	0.726	0.200	0.748
Sig.	Ns	<.001	<.001	Ns	Ns

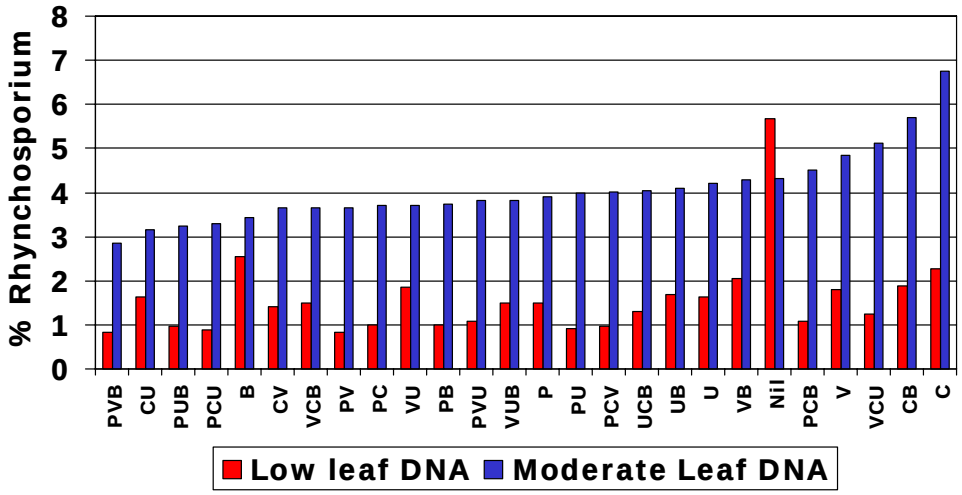

Figure 11 Eradicant activity against rhynchosporium at 28 days after treatment at different DNA thresholds

Rhynchosporium control was a greater challenge where higher levels of rhynchosporium DNA were present at the time of treatment (Figure 11). This results links well to visual disease, suggesting that testing leaves for rhynchosporium DNA can be a good predictor of the risk of disease pressure. The results are however confounded with sowing date, since winter sown crops had higher DNA levels at the time of treatment than spring sown crops. Despite a low DNA level, the untreated shows that under ideal weather conditions, there remains a high risk of disease development. The test may only determine the amount of fungicide required to deal with the risk.

Rhynchosporium secalis DNA

Rhynchosporium DNA was measured on final leaf 2 at the time of the overspray. Tables 23 and 24 show the DNA levels. DNA levels are likely to be affected by levels of visual disease. (Tables 23, 24 and Figure 12). Some of the less effective mixtures showed higher levels of DNA and this may correspond to the presence of symptoms on the leaves. Cyprodinil (U) gave different results on the winter and spring crop and levels were higher in spring barley where this fungicide was used. Mixtures comprising three fungicides generally achieved lowest DNA levels. This corresponds to the effective disease control with these mixtures. One exception to this pattern was with PUB in winter barley.

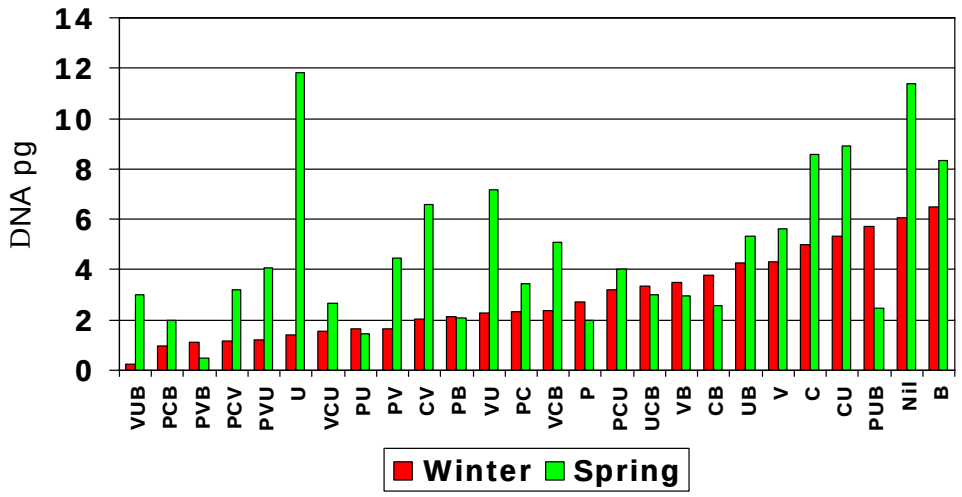

Figure 12 Leaf DNA at time of overspray

Table 23 Rhynchosporium DNA on final leaf 2 (post treatment), transformed amount (log(pg + 0.5)) from 2005-2007 trials

Sowing	Winter		Spring	
	1.53		1.81	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	2.38	1.97	1.70	1.48
Winter	2.09	1.74	1.56	1.34
Spring	2.60	2.15	1.81	1.58

	Weighted Mean	Winter	Spring
Nil	2.38	2.09	2.60
P	1.45	1.55	1.38
C	2.18	1.94	2.36
V	1.95	1.84	2.03
U	2.02	1.22	2.62
B	2.25	2.14	2.33
PC	1.60	1.47	1.70
PV	1.62	1.30	1.87
PU	1.26	1.29	1.23
PB	1.41	1.42	1.41
CV	1.83	1.40	2.15
CU	2.22	1.99	2.39
CB	1.62	1.76	1.52
VU	1.89	1.46	2.22
VB	1.64	1.70	1.60
UB	1.93	1.84	1.99
PCV	1.44	1.16	1.64
PCU	1.74	1.65	1.80
PCB	1.25	1.08	1.38
PVU	1.53	1.16	1.80
PVB	1.01	1.13	0.92
PUB	1.73	2.05	1.50
VCU	1.42	1.27	1.54
VCB	1.75	1.48	1.96
VUB	1.27	0.80	1.61
UCB	1.64	1.68	1.61

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	0.571	0.252	0.679	0.339	0.743
Sig.	Ns	<.001	Ns	Ns	Ns

Table 24 Rhynchosporium DNA on final leaf 2 (post treatment) pg DNA (back-transformed of Treatment x Sowing means from analysis)

	Winter	Spring
Nil	6.06	11.40
P	2.69	1.99
C	4.98	8.56
V	4.30	5.61
U	1.40	11.80
B	6.47	8.32
PC	2.34	3.46
PV	1.66	4.46
PU	1.64	1.43
PB	2.12	2.09
CV	2.05	6.58
CU	5.32	8.90
CB	3.79	2.58
VU	2.30	7.16
VB	3.49	2.94
UB	4.27	5.34
PCV	1.17	3.18
PCU	3.20	4.04
PCB	0.95	1.99
PVU	1.19	4.08
PVB	1.10	0.50
PUB	5.74	2.47
VCU	1.55	2.68
VCB	2.38	5.07
VUB	0.24	3.01
UCB	3.34	3.00

Impact of fungicide programmes based on late-season levels of DNA

The purpose of this analysis is to determine the response to fungicide where visual symptoms are low but where DNA levels are high at the end of the season. The hypothesis to be tested is that the highest response to fungicide is found where disease symptoms appear, but a high response to fungicide is also seen where *R secalis* DNA is present in the absence of symptoms.

Visual symptoms were greater in wet spring conditions. These trials should therefore respond most to fungicide, particularly in trials where *R secalis DNA* levels were also high (highest disease pressure). In low disease pressure trials (dry spring), where PCR levels were low, the response to fungicide should be lowest, since there would be the lowest levels of visual disease and DNA present in the crop (Table 25 & 26).

Table 25 Yield (t/ha) under different disease pressures in spring and different levels of rhynchosporium DNA late in season

	Dry spring Low DNA	**Dry spring High DNA**	**Wet spring Low DNA**	**Wet spring High DNA**
	6.14	7.57	6.99	8.10
	Number of Fungicides in Mixture			
	Nil	**1**	**2**	**3**
Weighted Mean	7.06	7.25	7.46	7.55
Dry spring Low DNA	5.96	6.07	6.17	6.17
Dry spring High DNA	7.04	7.33	7.55	7.76
Wet spring Low DNA	6.92	6.85	7.02	7.04
Wet spring High DNA	7.54	7.86	8.13	8.26

Table 26 Yield (t/ha) under different disease pressures in Spring and different levels of rhynchosporium DNA late in season

	Dry spring Low DNA	Dry spring High DNA	Wet spring Low DNA	Wet spring High DNA
Nil	5.96	7.04	6.92	7.54
P	6.19	7.86	6.92	8.11
C	6.05	7.46	6.85	7.71
V	6.15	7.09	6.87	7.85
U	5.94	7.35	6.78	7.94
B	6.03	6.91	6.82	7.71
PC	6.00	7.29	7.23	8.25
PV	6.35	7.95	7.07	8.35
PU	5.95	7.84	6.99	8.36
PB	6.29	7.81	6.77	8.34
CV	6.32	7.34	7.16	7.96
CU	6.18	7.72	7.08	7.82
CB	6.38	7.42	6.88	7.99
VU	6.06	7.36	7.13	7.89
VB	6.06	7.43	6.87	8.18
UB	6.10	7.31	7.06	8.18
PCV	6.06	7.92	6.92	8.25
PCU	6.27	7.96	7.04	8.25
PCB	6.28	7.90	7.05	8.28
PVU	6.21	7.87	7.09	8.31
PVB	6.45	7.53	7.04	8.58
PUB	5.90	7.92	7.21	8.42
VCU	6.151	7.786	7.086	8.142
VCB	6.312	7.710	6.935	8.200
VUB	6.060	7.747	7.114	7.973
UCB	5.982	7.200	6.922	8.157

	Spring rain, Late leaf DNA	Number	Spring rain, late leaf DNA x Number	Treatment	Spring rain, late Leaf DNA Treatment
Av. SED	1.035	0.074	1.052	0.099	
Sig.	Ns	<.001	0.010	<.001	

Table 27 shows the observed yield response and the visual disease in the Nil treatment. The highest yield responses were observed where DNA levels were higher in the leaves late in the season. The DNA levels did not however correlate well to visual disease.

Table 27 Yield response based on spring weather and leaf DNA levels.

Category	Expected Yield Response	Observed Yield Response (%)			Rhynchosporium (%) - Nil treatment
		Number			
		1	2	3	
Dry spring Low DNA	Low	2	4	3	6.6
Dry spring High DNA	High	4	7	10	1.8
Wet spring Low DNA	High	-1	2	2	1.8
Wet spring High DNA	Highest	4	8	10	12.1
Weighted Mean		3	6	7	

Green leaf area retention

Fungicides can also impact on green leaf area in positive and negative ways. Tables 28 and 29 show the green leaf retention for the fungicide mixtures 28 days after treatment. Green leaf area levels increased with fungicide dose, suggesting no major problem with phytotoxicity. Spring barley was more susceptible to green leaf area loss where chlorothalonil (Bravo) was used alone.

Table 28 Green leaf area retention 28 days after treatment (transformed percentages, empirical logit), from 2005-2007

Sowing	Winter		Spring	
	4.51		2.65	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	2.49	3.15	3.41	3.51
Winter	3.85	4.36	4.54	4.62
Spring	1.64	2.39	2.71	2.82

	Weighted Mean	Winter	Spring
Nil	2.49	3.85	1.64
P	3.39	4.40	2.75
C	3.16	4.11	2.58
V	3.05	4.22	2.32
U	3.20	4.50	2.39
B	2.95	4.57	1.93
PC	3.58	4.63	2.92
PV	3.62	4.64	2.99
PU	3.55	4.63	2.87
PB	3.50	4.59	2.82
CV	3.34	4.45	2.65
CU	3.35	4.50	2.62
CB	3.33	4.52	2.59
VU	3.25	4.49	2.47
VB	3.21	4.30	2.52
UB	3.42	4.70	2.62
PCV	3.49	4.49	2.86
PCU	3.66	4.80	2.95
PCB	3.50	4.53	2.86
PVU	3.45	4.41	2.85
PVB	3.59	4.68	2.90
PUB	3.51	4.76	2.74
VCU	3.56	4.61	2.91
VCB	3.58	4.64	2.92
VUB	3.41	4.75	2.57
UCB	3.36	4.58	2.60

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	1.208	0.133	1.225	0.180	1.235
Sig.	Ns	<.001	Ns	Ns	Ns

Table 29 Green leaf area retention 28 days after treatment (% leaf area - back-transformed of Treatment x Sowing means from analysis)

	Winter	Spring
Nil	98.4	84.1
P	99.3	94.4
C	98.9	93.4
V	99.0	91.4
U	99.4	92.0
B	99.5	87.7
PC	99.5	95.3
PV	99.5	95.7
PU	99.5	95.1
PB	99.5	94.8
CV	99.3	93.8
CU	99.4	93.7
CB	99.4	93.5
VU	99.4	92.6
VB	99.1	93.0
UB	99.6	93.7
PCV	99.4	95.0
PCU	99.7	95.5
PCB	99.4	95.0
PVU	99.3	95.0
PVB	99.6	95.2
PUB	99.6	94.4
VCU	99.5	95.3
VCB	99.5	95.4
VUB	99.6	93.3
UCB	99.5	93.5

Tables 30 and 31 and Fig. 13 show the green leaf area levels at GS55. The benefit from the three-way mixtures occurred in both winter and spring barley

Table 30 Green leaf area retention at time of overspray (transformed percentages, empirical logit), from 2005-2007

Sowing	Winter		Spring	
	2.53		1.19	
	Number of Fungicides in Mixture			
	Nil	1	2	3
Weighted Mean	0.94	1.50	1.79	1.95
Winter	1.81	2.39	2.55	2.65
Spring	0.29	0.83	1.23	1.43

Treatment	Weighted Mean	Winter	Spring
Nil	0.94	1.81	0.29
P	1.87	2.64	1.29
C	1.48	2.40	0.80
V	1.50	2.27	0.93
U	1.42	2.44	0.65
B	1.22	2.19	0.50
PC	1.87	2.68	1.27
PV	2.17	2.91	1.62
PU	2.10	2.74	1.63
PB	2.10	2.78	1.59
CV	1.71	2.40	1.19
CU	1.52	2.08	1.11
CB	1.62	2.26	1.14
VU	1.68	2.70	0.92
VB	1.64	2.57	0.95
UB	1.50	2.38	0.83
PCV	2.20	2.86	1.71
PCU	1.98	2.73	1.42
PCB	2.16	2.82	1.66
PVU	1.94	2.90	1.22
PVB	2.15	2.70	1.73
PUB	1.85	2.69	1.22
VCU	1.79	2.50	1.26
VCB	1.97	2.55	1.54
VUB	1.59	2.25	1.10
UCB	1.90	2.51	1.44

	Sowing	Number	Sowing x Number	Treatment	Sowing x Treatment
Av. SED	1.087	0.131	1.103	0.176	1.114
Sig.	Ns	<.001	0.10	<.001	Ns

Table 31 Green leaf area retention at time of overspray, % leaf area (back-transformed of Treatment x Sowing means from analysis)

	Winter	Spring
Nil	86.3	57.2
P	93.8	78.7
C	92.1	69.1
V	91.0	72.0
U	92.4	65.9
B	90.4	62.3
PC	94.0	78.3
PV	95.3	83.8
PU	94.3	83.9
PB	94.6	83.4
CV	92.1	77.0
CU	89.2	75.5
CB	91.0	76.1
VU	94.1	71.8
VB	93.3	72.3
UB	92.0	69.8
PCV	95.0	85.1
PCU	94.3	80.9
PCB	94.8	84.4
PVU	95.2	77.5
PVB	94.2	85.3
PUB	94.1	77.4
VCU	92.8	78.2
VCB	93.2	82.6
VUB	90.9	75.2
UCB	92.9	81.1

Two and three-way mixtures gave the best green leaf area retention. Prothioconazole was the best single fungicide, whilst chlorothalonil (B) or cyprodinil (U) or fenpropimorph (C) used alone achieving the lowest levels.

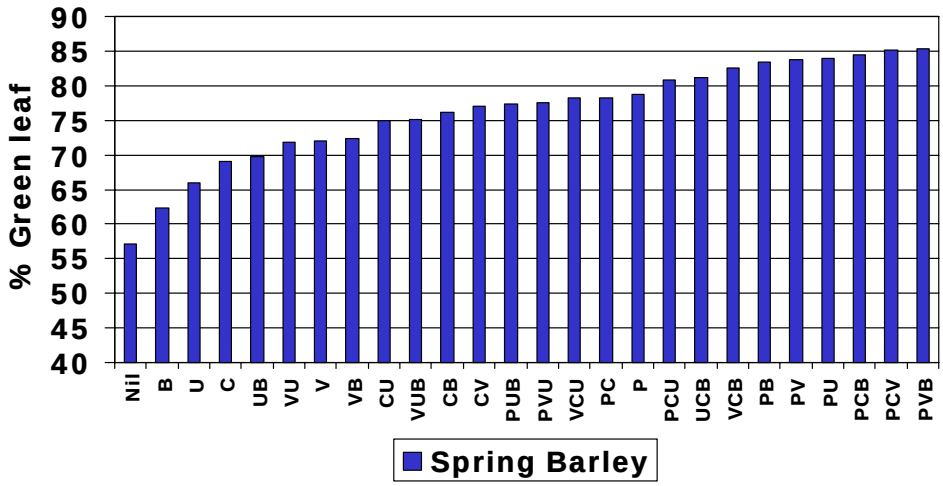

Figure 13. Green leaf area at GS55 in spring barley

Discussion

Field trials were carried out over three seasons at different sites to demonstrate fungicide performance in a range of scenarios. Winter and spring barley exhibited different disease pressures and yield responses to fungicide mixtures. There were also high and low disease pressure sites which allowed the trials to be categorised into a range of scenarios relevant to winter and spring barley growers.

Understanding the profitability of a fungicide programme when disease levels turn out to be low at the end of the season may influence the decision growers make in situations where rhynchosporium is not endemic every year and improve their confidence to take risks not to treat a crop. Taking decisions on using fungicides can be more challenging in low disease sites compared to regions where the disease is more endemic.

Initial analysis of results on fungicide resistance enabled further differentiation of the sites since rhynchosporium at some sites exhibited higher resistance to DMI (triazole) fungicides. Extrapolating the results from *in vitro* resistance studies to the fungicide performance in the field can be challenging, but the results from this research show that even in “resistant hot spots” for the triazole fungicide prothioconazole, this fungicide still remains the most effective active ingredient.

The triazole fungicide prothioconazole plays a major role in the management of rhynchosporium in winter and spring barley. When used alone, it achieved the best yield in winter and spring barley compared to the other fungicide active ingredients used alone. This agrees with results from HGCA funded Appropriate Fungicide Dose research (Oxley & Hunter 2005). This fungicide achieved the best margin over fungicide cost in spring barley and equalled the best margin in winter barley with cyprodinil (U) when comparing the single fungicide components. Prothioconazole also gave the best disease eradication and protection in high disease pressure situations and also in the triazole resistant sites compared to the other single fungicide active ingredients. Better disease control, yields and margins were attained from fungicide mixtures than from use of single active ingredients, but prothioconazole is a key component of two and three-way mixtures.

Two factors which may impact on the future use of the triazole fungicides are fungicide resistance and EU legislation. The risk of resistance is covered in detail in the next section of this report. On the legislative side, a review of the “The Plant Protection Products Directive” (91/414/EEC) may impact on the longer term use of triazole fungicides since this group of fungicides may fit the category of an active substance "candidate for substitution". The review currently states that fungicides within this category will not be approved for more than 7 years if other substances on the market present "significantly lower risk“. Although this research highlights the importance of this fungicide group in managing rhynchosporium in barley, if it were to disappear, alternative fungicide mixtures could be selected from this research and compared for profitability. The loss of a second fungicide group, for example strobilurin fungicides as a consequence of resistance, would have a catastrophic impact on our ability to manage disease on the basis of current varieties and in the absence of new fungicide active ingredients.

The “Nil” treatment, which received a late fungicide only, gave the lowest yield in both winter and spring barley. Increasing the number of components in a fungicide mixture led to an increase in yield in both winter and spring barley. Three-way mixtures were better than two-way mixtures and two-way mixtures better than a single fungicide component. Although this result is what would be expected, occasionally an increase in dose can lead to phytotoxic effects on the crop and limit yield: this was not observed at the fungicide timings used in the trials (GS25-32).

At the start of this research in 2004, grain prices were in the region of £75/tonne. By 2008, prices had doubled in price. Margins over fungicide cost have been calculated at grain prices of £75, £125 and £175/tonne to determine the impact grain price has on the fungicide mixture. Fungicide prices have increased by a factor of 5-8% over the period grain prices have doubled. Fungicide margins based on the low grain price show that fungicide mixtures comprising two active ingredients achieved the best margin in both winter and spring barley. Using a mixture with three components did however give a similar margin which was not significantly lower than the two component mixture.

In the current economic climate of grain prices at £175/t, the higher yields achieved from a three-way mixture in winter barley show that fungicide mixtures comprising three components is significantly more profitable than a two component mixture. For

spring barley, where yield response to fungicide is lower, two and three component mixtures were similar. There is no evidence from this research to suggest that cutting fungicide mixtures to a single component is economic at any grain prices. This is an important message both to the grower to get the best margin and also important in reducing the risk of fungicide resistance.

Winter barley requires effective eradication of rhynchosporium at GS31-32, since the disease may be already established inside the crop. This can be determined either through visual assessment or using a molecular test which measures rhynchosporium DNA. In spring barley, disease was not seen at time of treatment, and rhynchosporium DNA levels were low. The results from this research show that walking crops is a good method to determine the risk of disease. Using a molecular test is also effective and may be important indicator of disease risk where symptoms have yet to develop. The use of the diagnostic is discussed further later in this section.

Eradication of and protection from rhynchosporium requires different characteristics of a fungicide mixture. Eradicant activity was determined by measuring disease on the lower leaves 28 days after treatment. Protectant activity was determined by measuring disease on the top leaves at the time of the overspray. This was an average of 34 days after treatment in the spring barley and 38 days after treatment in the winter barley treatment.

A key component of a fungicide mixture for eradicant activity was pyraclostrobin (V). This fungicide did not however perform well alone or when mixed with chlorothalonil (VB). The best eradicant mixtures were pyraclostrobin + prothioconazole + chlorothalonil (PVB), pyraclostrobin + chlorothalonil + fenpropimorph (VCB), pyraclostrobin + cyprodinil (VU) and pyraclostrobin + prothioconazole (PV). Of these four treatments, the most cost effective were PV and PVB. With the exception of prothioconazole + chlorothalonil (PB), all two-way mixtures comprising chlorothalonil as one of the components were weak at eradicating rhynchosporium. This suggests chlorothalonil reduces the eradicant activity of the partner fungicide. The negative effect from chlorothalonil was not seen in the three-way mixtures. Focusing on the high disease pressure situations where weather was wet during the spring, additional fungicide mixtures for eradicant activity were prothioconazole + pyraclostrobin + fenpropimorph (PCV) and prothioconazole + cyprodinil + fenpropimorph (PCU). These

two mixtures comprise a morpholine component, which achieves poor control when used alone, but which is a useful component of a mixture in an eradicant situation.

In areas with high resistance to prothioconazole the three mixtures which achieved the best eradicant activity did not contain prothioconazole. These were pyraclostrobin + cyprodinil (VU), fenpropimorph + cyprodinil (CU) and pyraclostrobin + fenpropimorph + chlorothalonil (VCB). This result suggests cyprodinil plays an important part along with pyraclostrobin in these sites. Cyprodinil also contributes more to yield in winter barley than rhynchosporium activity alone would suggest. Cyprodinil will also control eyespot and control of this stem base disease may be the reason for the additional increase in yield.

Disease protection requires a longer period of persistence (30-40 days). The best single fungicide component was prothioconazole (P) followed by chlorothalonil (B). All other single fungicides gave the least control. Three-way mixtures were significantly better than two-way mixtures suggesting high doses, or three active ingredients achieved a longer period of persistence.

Prothioconazole and chlorothalonil were key components of mixtures to give the best protection. The strength from pyraclostrobin as a key for eradication was less obvious as a key component for protection.

Eradication of rhynchosporium is required most in winter barley at GS31-32, since the disease may be well established at the time of treatment. Visual assessment of the crop for disease is a good indicator of the disease pressure at time of treatment. If it can be seen, the potential risk is high and eradicant activity is required. The molecular diagnostic measures rhynchosporium DNA inside a leaf and higher levels of DNA were recorded in winter barley compared to spring barley at the time of treatment. The diagnostic test also correlates well with visual assessment. Both methods are therefore equally effective at determining the potential risk of disease. The molecular test would be a more accurate indicator, since it will determine the risk before symptoms appear.

High disease at time of treatment or high rhynchosporium DNA levels does not necessarily lead to high disease late in the season. Weather factors play a major role in symptom development later in the season. The 2007 season showed this well, since

DNA was present in the leaves and shoots, but the dry April and May weather subsequently led to a slow build up of disease late in the season, despite a cool and wet June and July.

DNA levels were also tested on the late leaves and the lowest DNA levels were seen in the three component mixtures and most in the untreated and the single fungicide components.

Trials were also categorised by spring rainfall, which was a high risk factor for late disease development and by the amount of DNA late in the season. It was hypothesised that highest yield benefits from fungicides would be achieved in the wet spring where DNA levels were high and lowest levels in a dry spring where late DNA levels were low. Results from the “Dry Spring High DNA” category showed the second highest increase in yield. This result suggests that in the absence of visual rhynchosporium on the crop, but with the presence of rhynchosporium DNA within the plant, fungicides can achieve a yield benefit. This observation suggests asymptomatic disease is of equal importance to visual presence of symptoms. This will be of great importance to plant breeders, since breeding for disease resistance needs to focus on the resistance of asymptomatic disease and not solely on symptom development alone. Diagnostic tools must therefore become a key tool for plant breeders to determine the presence of varietal resistance through a varieties capability to resist a fungicide developing inside a plant regardless the presence of visual symptoms. . Care has to be taken, however, since the categories may differentiate between winter and spring barley. It is however an interesting observation which requires more study.

Impact of fungicide mixtures and sequences on rhynchosporium sensitivity

Introduction

Field efficacy from fungicides is not stable and the development of fungicide resistance in pathogens across many fungicide groups is well documented for a range of cereal pathogens. Shifts in sensitivity can lead to failures in disease control and significant economic losses, both through the continued use of ineffective products and through yield losses as a result of poor disease control. *Rhynchosporium secalis* has, in the past, adapted to several of the fungicide groups used for control of leaf scald. Currently a range of chemical groups are used including the DMI group (e.g. prothioconazole, epoxiconazole), the anilinopyrimidine group (e.g. cyprodinil), MBC (e.g. carbendazim), morpholines (e.g. fenpropimorph) and strobilurins (e.g. pyraclostrobin).

Anti-resistance strategies have focused on the use of mixtures or alternations of fungicides. For efficacy reasons, fungicides for rhynchosporium control are applied in mixtures but if one or more of the current actives were to lose efficacy through resistance development, disease control would be compromised. A key aim of this section of the project was therefore to assess the sensitivity of *R. secalis* to these key groups and to determine if particular fungicide treatment combinations influenced the risk of resistance development. On the basis of the findings recommendations could be made to growers as to best practice to extend or maintain the effective life key fungicide groups.

Literature review

The risk of resistance developing in a given pathogen is dependent on several factors. The biology, abundance and stability of the pathogen is one factor in the ease with which it can mutate and then spread. The usage pattern of the active ingredient is a further factor with heavy usage increasing the selection pressure and competitive advantage for resistance. Finally, there is an inherent risk of resistance to any fungicide group - and a risk matrix (www.frac.info) is often used as a guide to the perceived risk, with fungicides effective at a single point in a biochemical pathway in the host judged to be most at risk and multi-site fungicides where many mutations in the pathogen would be required to confer resistance judged to be at lower risk.

Strobilurins, which inhibit a single site involved in mitochondrial electron transfer, are known to be at risk with well documented failures in disease control with several cereal pathogens including *Septoria tritici* on wheat, powdery mildew on both wheat and barley and net blotch on barley. A single mutation (G143A) is responsible for conferring complete resistance on all these pathogens, except for net blotch, where a different mutation giving a lower level of resistance has developed (Heaney *et al.* 2000; Gisi *et al*., 2002). There are therefore two alternative modes of resistance to strobilurins that are known and nothing to prevent other alternative mutations also conferring resistance.

One of the key fungicide groups for control of *R. secalis* is the demethylation inhibitor (DMI) group colloquially known as azoles. The DMIs are part of a larger group that inhibit sterol biosynthesis - the SBI group that also includes the morpholines and spiroxamine. In a previous HGCA report Cooke and Locke (2002) concluded that sensitivity to the DMI group was highly variable and that selection and shifts in sensitivity were still ongoing. Reduced sensitivity is under the control of multiple genes and / or multiple mutations hence the gradual, multi-step resistance observed, in contrast to groups like the strobilurins where resistance is conferred by a single gene mutation and the resistance seen is discrete. Cooke and Locke (2002) and Cooke *et al*., (2004) recommended that DMIs always be supplemented by a partner fungicide from a group with a different mode of action. At that time the most effective partners were from the strobilurin (QoI) or anilinopyrimidine groups in terms of disease control and yield. Their work also concluded that the use of effective partners in this way not only improved efficacy but also helped to reduce selection for DMI resistance, but did not prevent it.

Cooke and Locke (2002) made the following recommendations:-

- To avoid using DMIs as sole active ingredients and particularly avoid repeated use of half-rates of DMIs alone.
- In some regions, DMIs might no longer be worthwhile components of *R. secalis* control programmes.
- That reliance on strobilurin or anilinopyrimidine fungicides alone also posed potential risks of resistance.

DMI fungicides have been used on barley since the 1970s, initially for the control of mildew which rapidly developed resistance to the fungicide group (e.g. Wolfe, 1984; Heaney *et al*., 1986; Clark, 1992). Subsequently DMIs were used in mixtures with fungicides with different modes of action. The development of reduced sensitivity in *R. secalis* has been recorded since the mid 1980s (Kendall *et al*., 1993) and DMIs were therefore used in mixture with benzimidazoles for effective control and as an anti-resistance strategy. Benzimidazole resistance in *R. secalis* developed at the start of the 1990s and subsequently became both common and widespread (Taggart *et al.*, 1998; Taggart *et al*., 1999). The use of DMI fungicides in mixtures with morpholine fungicides and, from the mid-1990s, with strobilurins and anilinopyrimidines provided fungicide mixtures with alternative modes of action and potentially reduced the selection pressure on the individual fungicide groups. By the mid 2000s chlorothalonil was also commonly used in control programmes. The introduction of the related DMI prothioconazole in 2004 improved disease control and it quickly became the product of choice in barley programmes. This reliance on a product in control programmes clearly increases the risk of resistance developing and how best to steward this and other key actives was a major aim of the project.

Fungicides from the morpholine group are also used in *R. secalis* control programmes particularly where eradicant activity is desired. The morpholines are part of the sterol biosynthesis group (SBI) along with the DMIs and are classed as being at slightly lower risk as they are known to be active at least two points in the biochemical pathway, both distinct from the target site of the DMIs. There are documented cases of reduced and variable sensitivity in powdery mildews on wheat and barley.

Cyprodinil is a common mixing partner to other fungicides on barley. Part of the anilinopyrimidine group of fungicides, resistant isolates have been reported in several orchard and vineyard pathogens (www.FRAC.info), but there has been only one field report of resistance in a cereal pathogen with isolates of the eyespot causal organism *Oculimacula yallundae* and *O. acuformis* found with reduced sensitivity to cyprodinil (Babij et al. 2000). There is therefore a demonstrable risk of resistance in this active group also.

Aims

The aim of this section of the work was to establish the sensitivity of *R. secalis* to the key fungicide groups used for control of leaf scald in barley and further to determine if

different usage patterns and mixtures influenced the risk of resistance developing. The goal was to deliver clear usage strategy advice to growers that would effectively preserve the useful life-span of the most effective products.

Materials and Methods

Objective: To determine the sensitivity of *Rhynchosporium secalis* to fungicides *in vitro*

Procedure:

A. Isolation of *Rhynchosporium secalis* using antibiotic MYG (Malt yeast glucose) agar amended with iprodione

Before fungicide treatments were applied, leaves with lesions of *R. secalis* were collected from the three control plots and from three pre-treatment plots to allow the initial sensitivity of the *R. secalis* population at each site to be determined. Around 20-30 days after fungicide application and prior to over treatment of plots with Bravo, 50-100 lesion-bearing leaves were collected from each plot. The leaves were air-dried at room temperature and stored in a –20 °C freezer to produce one mixed isolate per sampled plot.

Active *Rhynchosporium secalis* lesions were cut from leaf segments using scissors, leaving a little green leaf area around the lesion. Lesions were then washed by submersing them in SDW (sterile distilled water) for 10 minutes. In a laminar flow cabinet, leaves were surface sterilised by submersing in 10 % sodium hypochlorite for 1.5 minutes and then allowed to dry on filter paper for 5 minutes.

Segments were then plated (5-6 lesions per plate) onto antibiotic MYG agar (1 litre distilled water with 10 g yeast, 6 g agar, 10 g malt, 10 g glucose, 0.1 g chloramphenicol) amended with iprodione (10 mg/l) ensuring that the lesion was uppermost on the leaf surface and dishes were sealed with parafilm. The inverted dishes were then incubated at 18 °C for up to three weeks.

Starting three days after plates were set up, they were examined daily for development of *R. secalis* until growth was noted when the top of the growing cultures was picked off under sterile conditions and plated onto fresh potato dextrose agar (PDA: 39 g in 1 litre of distilled water).

B. **Sensitivity testing in liquid medium**

Spore suspensions (20 µl) of test isolates were added to each well of 96-well Petri plates. Glucose-gelatin broth (4 g glucose, 4 g gelatine, 1.7 g KH_2PO_4, 0.75 g $MgSO_4$ in 1 litre of distilled water), amended with fungicide to achieve final concentrations of 100, 10, 1, 0.1, 0.01, 0.001 and 0 mg of active ingredient of the test fungicide/litre, was then pipetted into each well (180 µl per well). The fungicides tested were epoxiconazole (as Opus), prothioconazole (as Proline), fluoxastrobin (technical grade), pyraclostrobin (as Vivid), cyprodinil (as Unix) and fenpropimorph (as Corbel). Isolates collected each season were tested for sensitivity to epoxiconazole, prothioconazole, pyraclostrobin and fluoxastrobin. Isolates were only tested for sensitivity to fenpropimorph in 2007 and for cyprodinil in 2005 and 2006. Each isolate was tested in three replicate wells. Plates were measured for optical density (absorbance at 450 nm) on a Labsystems Multiskan plate reader initially and again after incubation (14 days in dark, 19°C with gentle rocking). From the subsequent differences the ED_{50} values were calculated in Genstat 10.2.

Results

R. secalis sensitivity to fungicides

The median, mean and ranges in sensitivity of the isolates are shown (Table 1) as ED_{50} values expressed in mg/l.

Table 32. ED_{50} values for isolates of *R. secalis* tested

Fungicide	Number tested	Median	Mean	Range	Range Q1- Q3
epoxiconazole	262	5.05	12.7	0.01-78.4	0.29-18.2
prothioconazole	240	5.06	13.4	0.01-100	0.21-17.5
fluoxastrobin	213	0.072	0.518	0.001-16.8	0.027-0.411
pyraclostrobin	230	0.062	0.376	0.001-18.0	0.005-0.198
cyprodinil	114	1.67	6.50	0.01-100	0.080-9.01
fenpropimorph	61	0.065	2.67	0.001-43.9	0.019-3.73

Q = quartile

The sensitivity data for the isolates to all products show an unusual distribution and, while the majority of isolates fall closer to the median values, there was a long distribution 'tail' to the least sensitive isolates assessed, as shown by product in the 'box and whisker' plots in Figure 13 The middle bar represents the median, the box the lower quartile or bottom 25 % to the upper quartile marks and the whisker is a measure of the points out with this upper to lower quartile range.

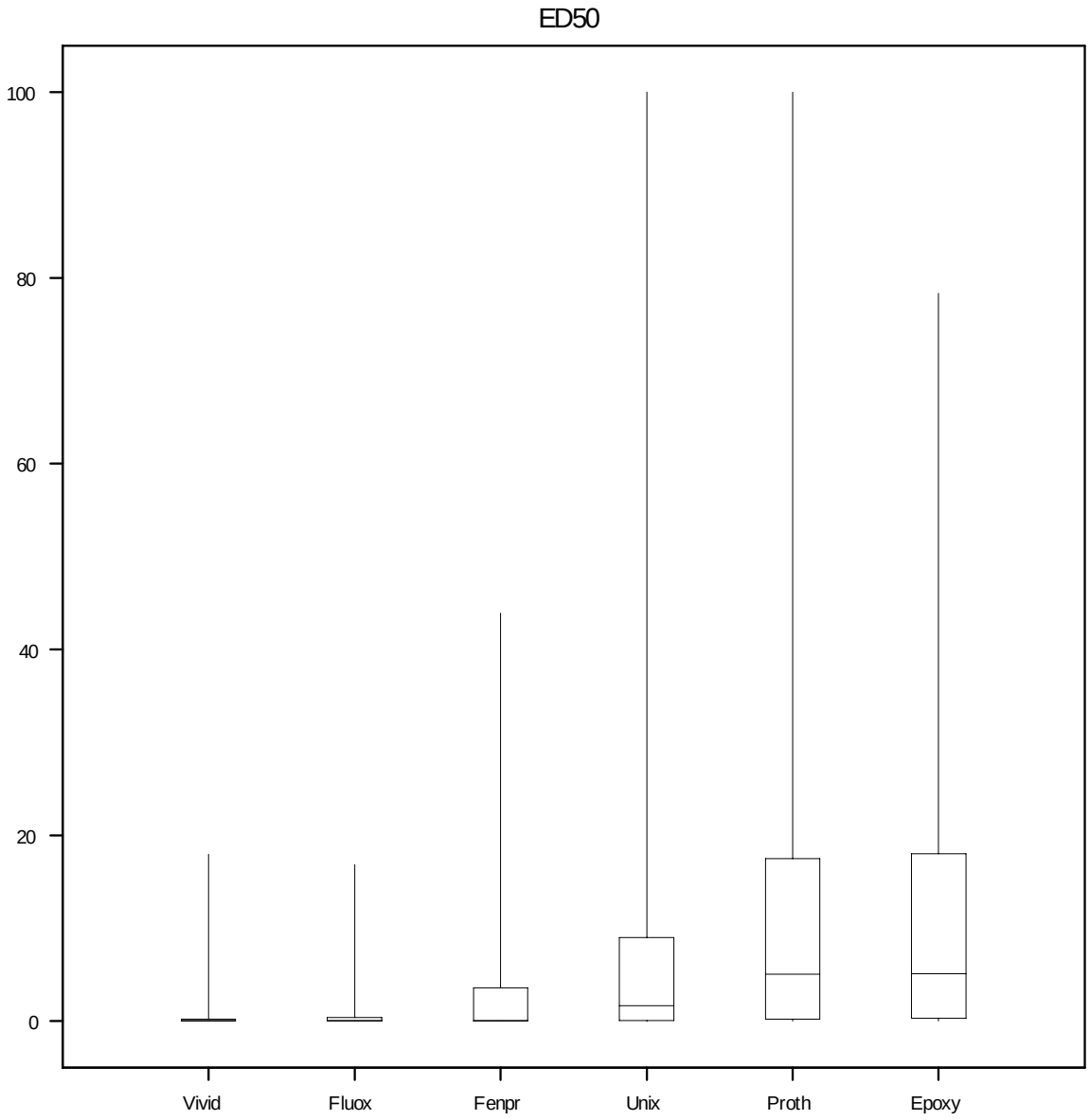

Figure 13. Sensitivity to test fungicides (Vivid), fluoxastrobin (Fluox), fenpropimorph (Fenpr), cyprodinil (Unix), prothioconazole (Proth) and epoxiconazole (Epox).

There was a wide range of sensitivity to epoxiconazole (Figure 14) - the median ED_{50} was 5.05 ppm. The range in sensitivity to both epoxiconazole and prothioconazole was wider than for the other fungicides tested. The measured sensitivity to prothioconazole fell broadly in the same range as epoxiconazole, with a similar pattern of distribution with a few isolates lying towards highly elevated ED_{50} values. It is not

possible to compare between or rank fungicides from these ED_{50} values generated from *in vitro* sensitivity tests and as they cannot represent the *in planta* efficacy.

In contrast to the two DMI fungicides, the majority of isolates had sensitivities to the strobilurin fungicides within a very small range from the median ED_{50} value with very few outlying isolates. These outliers have not been re-tested and could be artifacts of the test system. The isolates showed a similar level and range of sensitivities to pyraclostrobin as they did to fluoxastrobin with most isolates having values close to the median value. As with fluoxastrobin, there were a few outliers. These tended not to be those with measured high values to pyraclostrobin which would suggest they were artifacts of the testing system and the result would be unlikely to be repeated if they were re-tested.

Isolates were generally very sensitive to cyprodinil and the normal range was far less than that seen with the DMI fungicides tested. There were several outliers so that this fungicide also showed the skewed distribution seen for the other fungicides.

Fewer isolates were tested for sensitivity to fenpropimorph than were tested against other products. The ED_{50} values tend to be wider in range than was seen for the strobilurin products but not as wide as those seen for the DMI fungicides. As with other fungicides there was an unusual distribution with a few isolates lying far from the recorded median value for fenpropimorph.

Cross-resistance

Isolates with reduced sensitivity to prothioconazole also tended to have reduced sensitivity to epoxiconazole and similarly isolates that were sensitive to one of these SBI fungicides were likely to be sensitive to the other. There was a highly significant correlation (r=0.714, *P*<0.001) between the sensitivities of isolates to prothioconazole and epoxiconazole (Figure 15) indicating cross resistance.

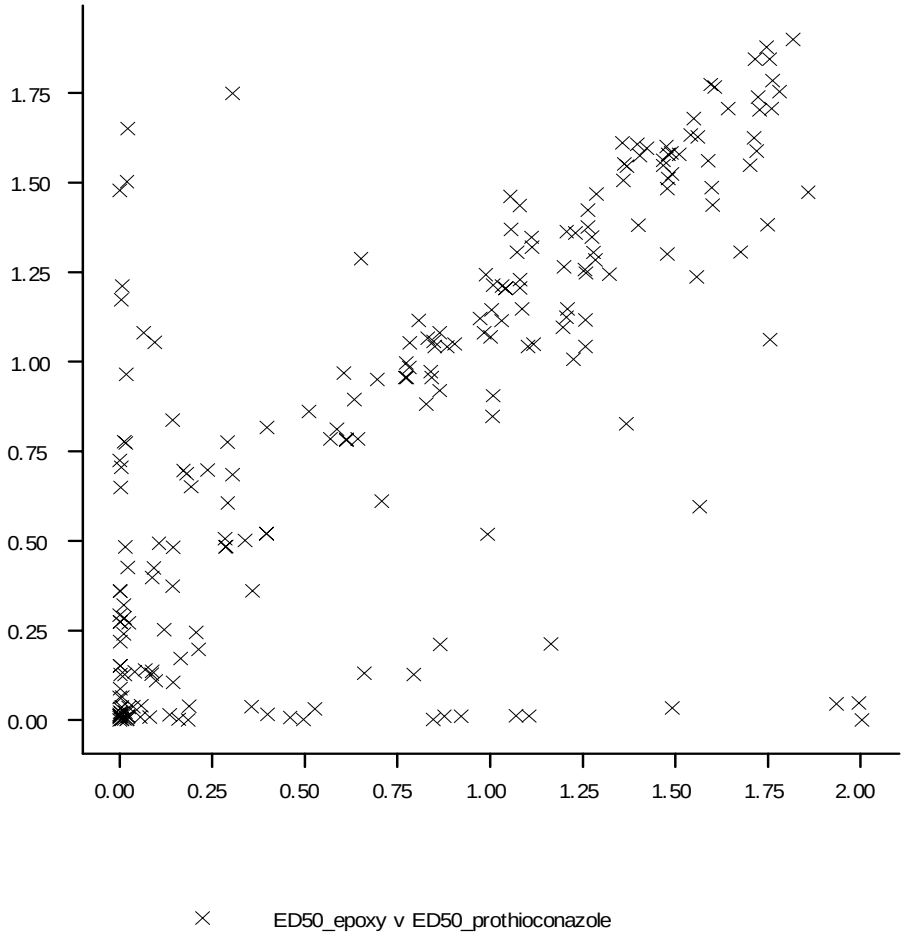

Figure 15. Sensitivity of *R. secalis* isolates to epoxiconazole and prothioconazole showing cross resistance (units as $\log^{10}(ED_{50}+1)$)

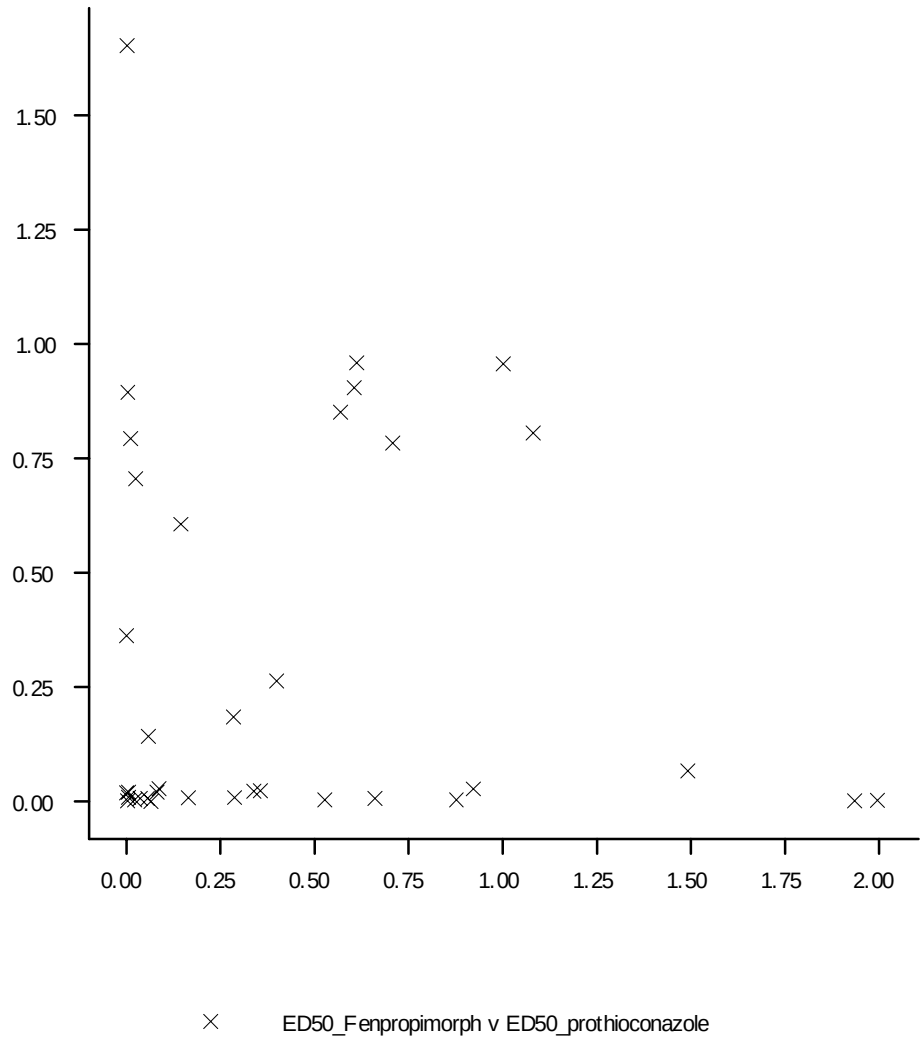

Figure 16. Sensitivity of *R. secalis* isolates to the SBI fungicides fenpropimorph and prothioconazole (units as $\log^{10}(ED_{50}+1)$)

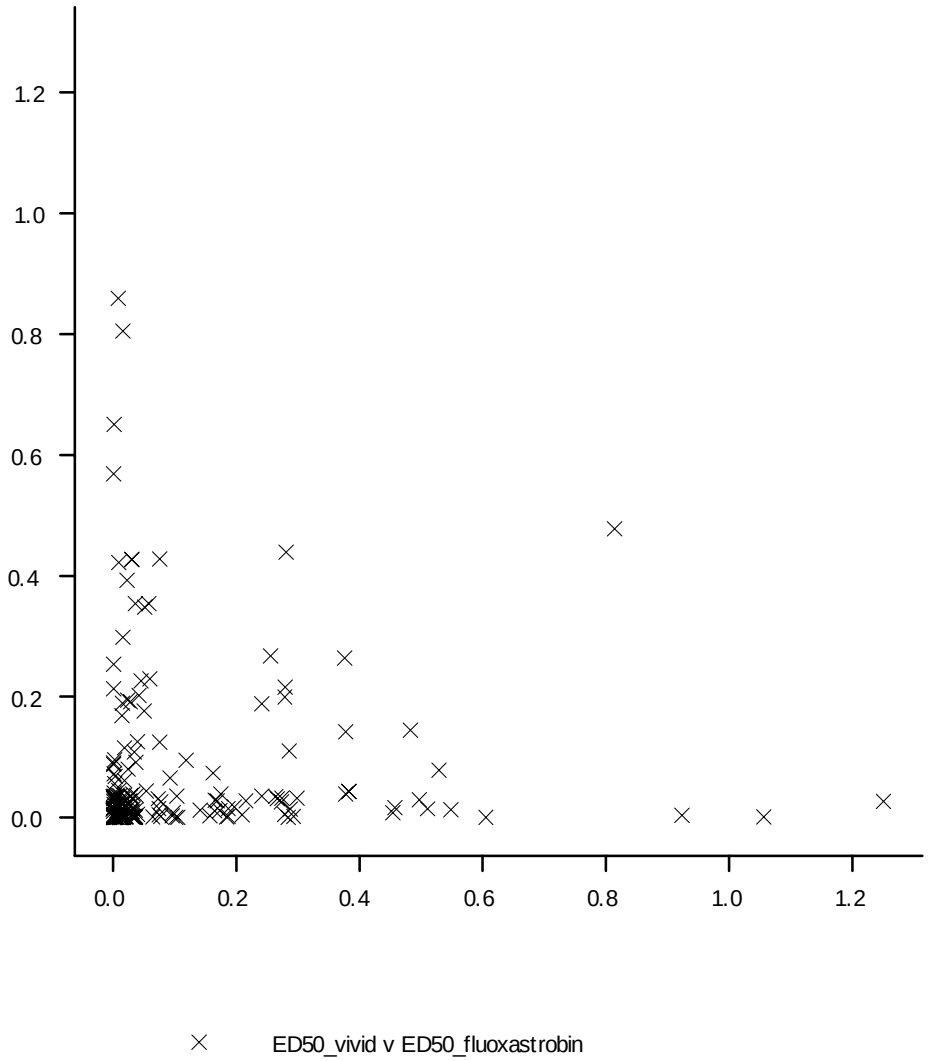

Figure 17. Sensitivity of *R. secalis* isolates to the strobilurin fungicides fluoxastrobin and pyraclostrobin (units as $\log^{10}(ED_{50}+1)$)

There was a lack of correlation between the two strobilurin fungicides (Figure 17) with the extreme ED_{50} values for one not coinciding with the extreme values for the other which implies that the outlying values are artifacts of the test system and are unlikely to be replicated in repeat testing.

Correlation coefficients for all products are shown in Table 33.

Table 33. Correlation co-efficients and significance for isolate sensitivity values

	epoxi-conazole	prothio-conazole	pyraclo-strobin	fluoxa-strobin	cyprodinil	fenprop-imorph
Epoxiconazole	-	0.714	-0.224	-0.070	-0.069	0.357
P value		<0.001	NS	NS	NS	0.02
Prothioconazole		-	-0.140	-0.018	-0.034	-0.040
P value			NS	NS	NS	NS
Pyraclostrobin			-	<-0.001	0.031	-0.032
P value				NS	NS	NS
Fluoxastrobin				-	0.179	-0.347
P value					NS	NS
cyprodinil					-	*
P value						

* no isolates tested against both active ingredients

There was no significant correlation between fenpropimorph and prothioconazole, however there was a weak correlation between fenpropimorph and epoxiconazole also in the SBI family of fungicides. On the limited number of isolates tested for sensitivity to fenpropimorph (61) this may not be representative of the larger population.

Variation by year

There were large variations in sensitivity by site but no indication of any drift or shift in sensitivity to the test fungicides over the duration of the project. (Figures 18-22).

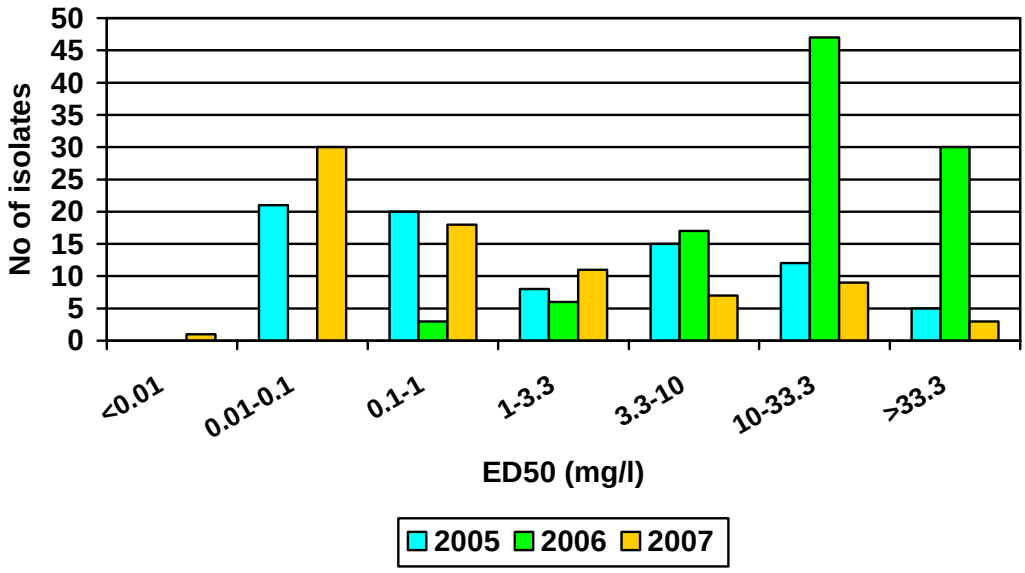

Figure 18. Sensitivity of *R. secalis* isolates to epoxiconazole between 2005 and 2007

There was an apparent shift in sensitivity to epoxiconazole ($P<0.001$) in 2006 but this shift was not evident in 2007 (Figure 18). Analysis of the sensitivities by site (shown later) indicates that this increase in ED_{50} values in 2006 was related to sampling from one particular site.

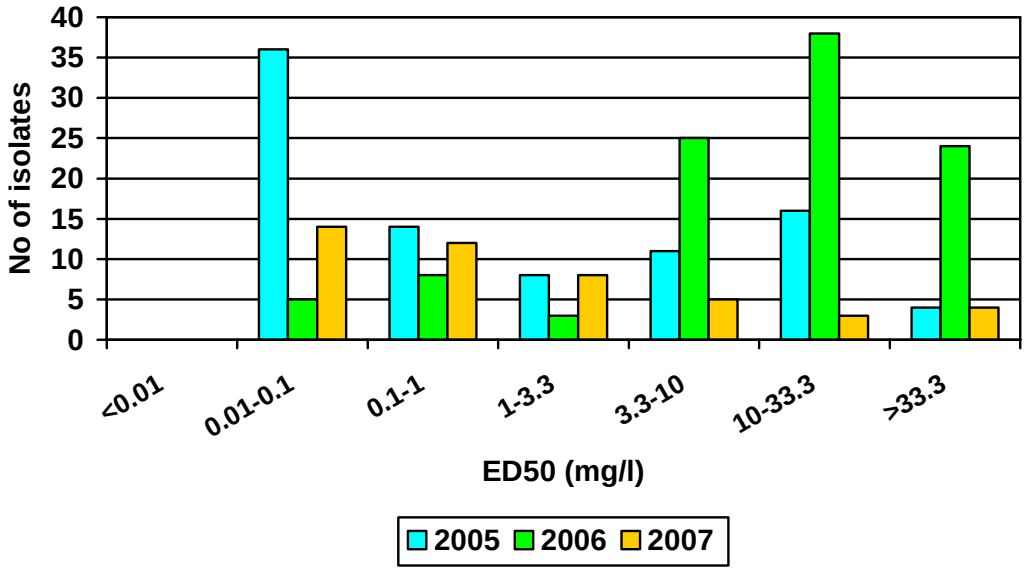

Figure 19. Sensitivity of *R. secalis* isolates to prothioconazole between 2005 and 2007

There was a comparable shift in prothioconazole sensitivity in 2006 ($P<0.001$) as there was to epoxiconazole and this can be explained by a site effect, with no evidence of any shift in sensitivity from the 2005 season to the 2007 season (Figure 19).

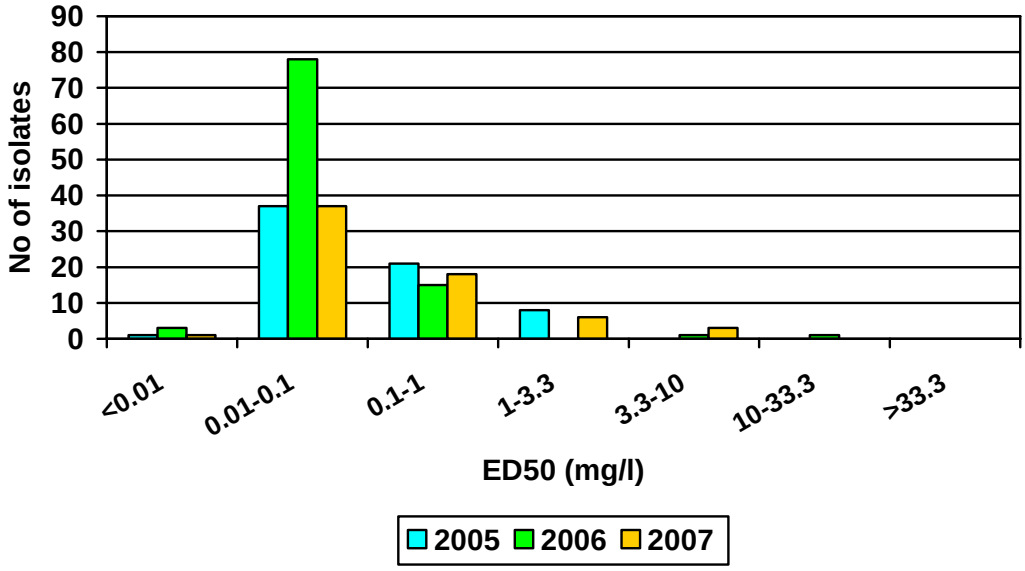

Figure 20. Sensitivity of *R. secalis* isolates to pyraclostrobin between 2005 and 2007

There was a no evidence of a shift in sensitivity to pyraclostrobin over the duration of the project with most isolates assessed as highly sensitive (Figure 20).

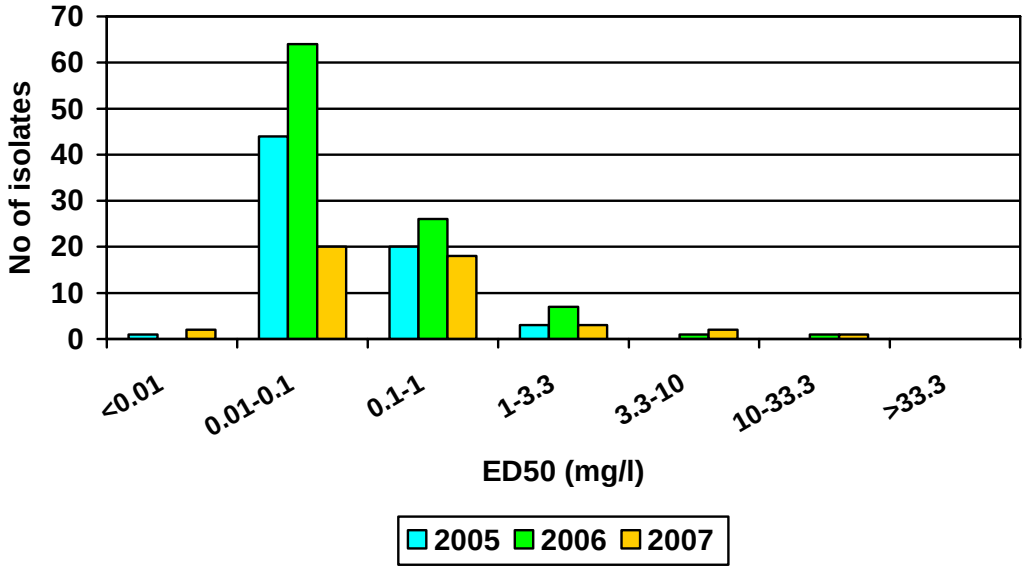

Figure 21. Sensitivity of *R. secalis* isolates to fluoxastrobin between 2005 and 2007

As with pyraclostrobin, there was no evidence of any shift between season in sensitivity to fluoxastrobin with most isolates highly sensitive to the strobilurin group (Figure 21).

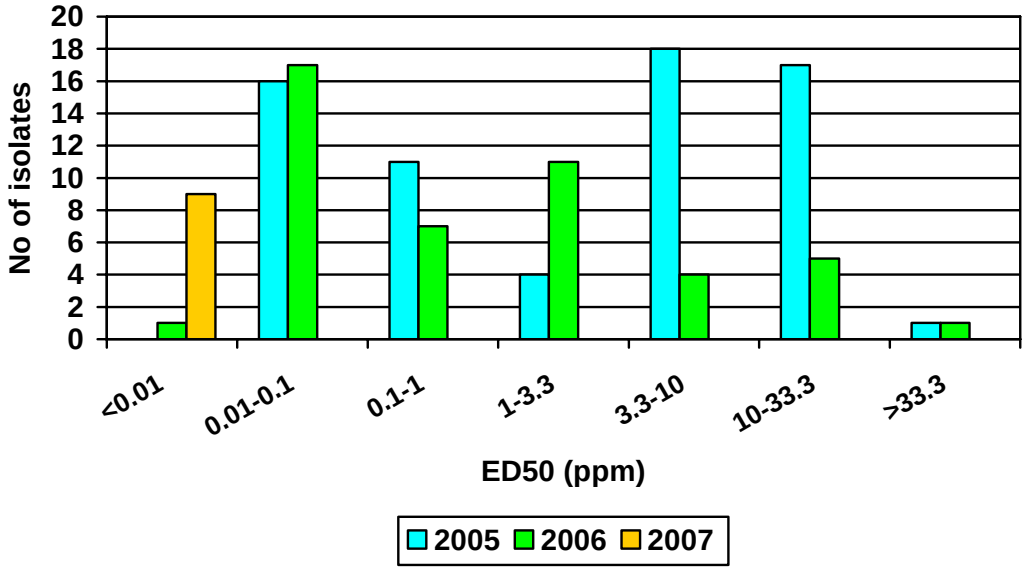

Figure 22. Sensitivity of *R. secalis* isolates to cyprodinil between 2005 and 2007

Isolates were only tested for sensitivity to cyprodinil in 2005 and 2006. There was no significant shift between these two seasons but there was a trend for isolates to be more sensitive in 2006 (Figure 22).

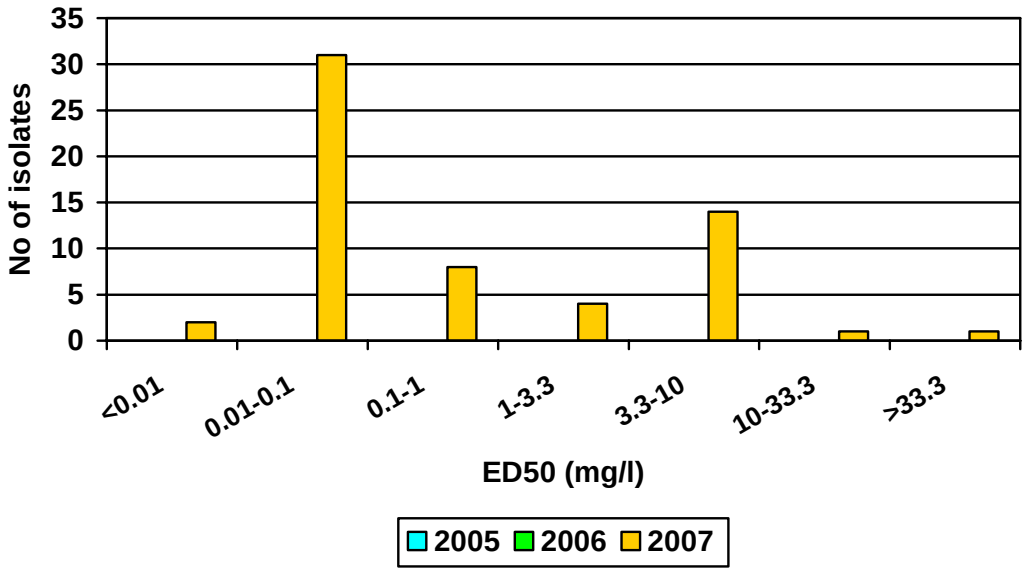

Figure 23. Sensitivity of *R. secalis* isolates to fenpropimorph in 2007

Isolates were only tested for sensitivity to fenpropimorph in 2007 - the distribution is shown above (Figure 23), with a wide range of sensitivities recorded.

Variation by site

There were variations in the sensitivities of isolates of *R. secalis* relative to the trial sites where they were sampled (Figures 24-29).

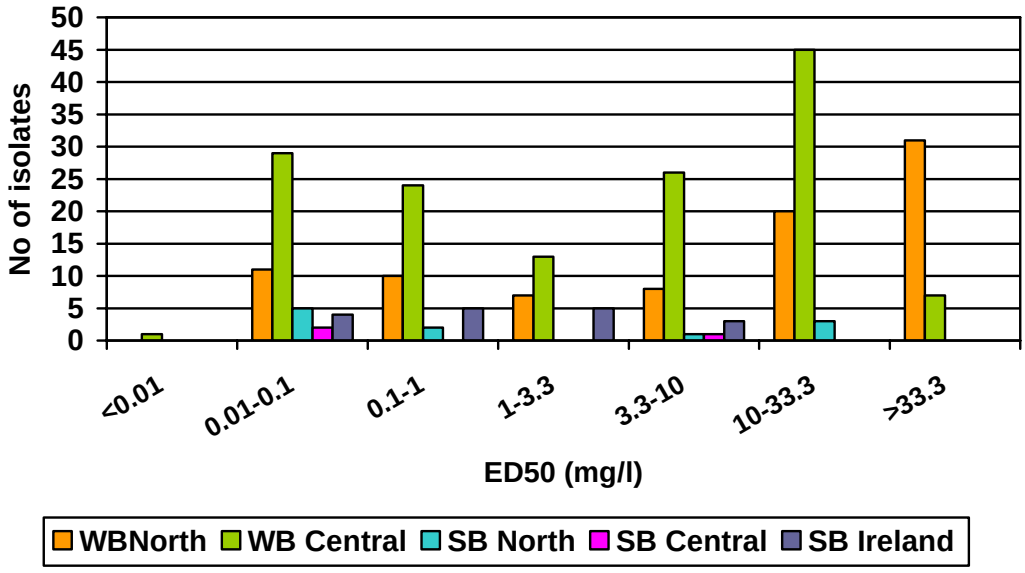

Figure 24. Sensitivity to epoxiconazole of *R. secalis* isolates from different trial locations.

There were significantly (P<0.001) more isolates with reduced sensitivity to epoxiconazole from the winter barley trials located in the north of Scotland than were found at other locations (Figure 24). Conversely there were no isolates in the least sensitive groupings from the trials located in Ireland - with isolates from these sites more sensitive than Scottish isolates. Disease levels tended to be lower in spring barley trials with fewer isolates of *R. secalis* isolated and tested as a result.

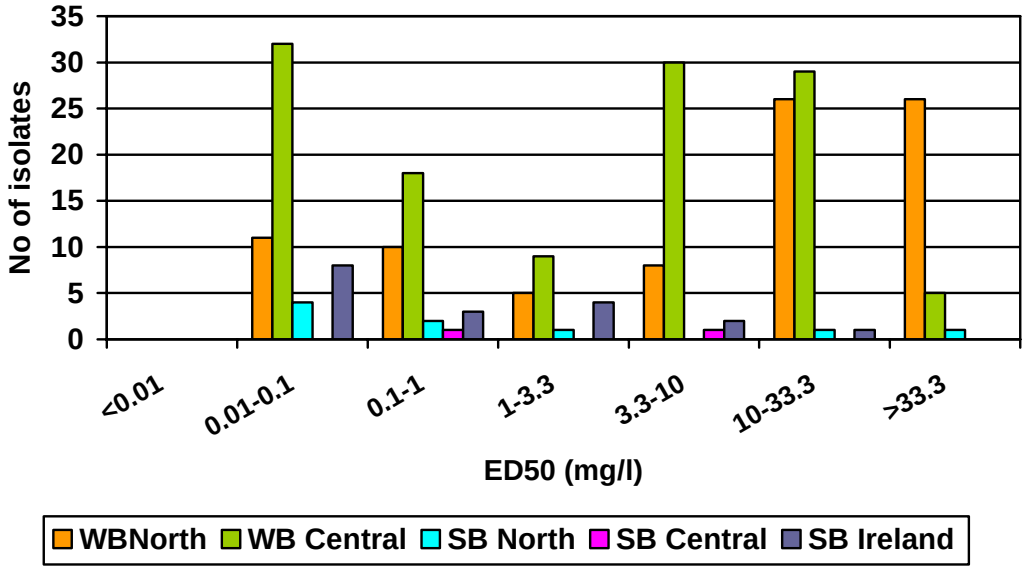

Figure 24. Sensitivity to prothioconazole of *R. secalis* isolates from different trial locations.

More isolates of *R. secalis* from the sites in the north of Scotland had higher ED_{50} values for prothioconazole (P<0.001) than those from other sites (Figure 25). As with sensitivity to epoxiconazole, none of the isolates collected from trials sites in Ireland had ED_{50} values in the higher categories and instead were grouped in the most sensitive categories.

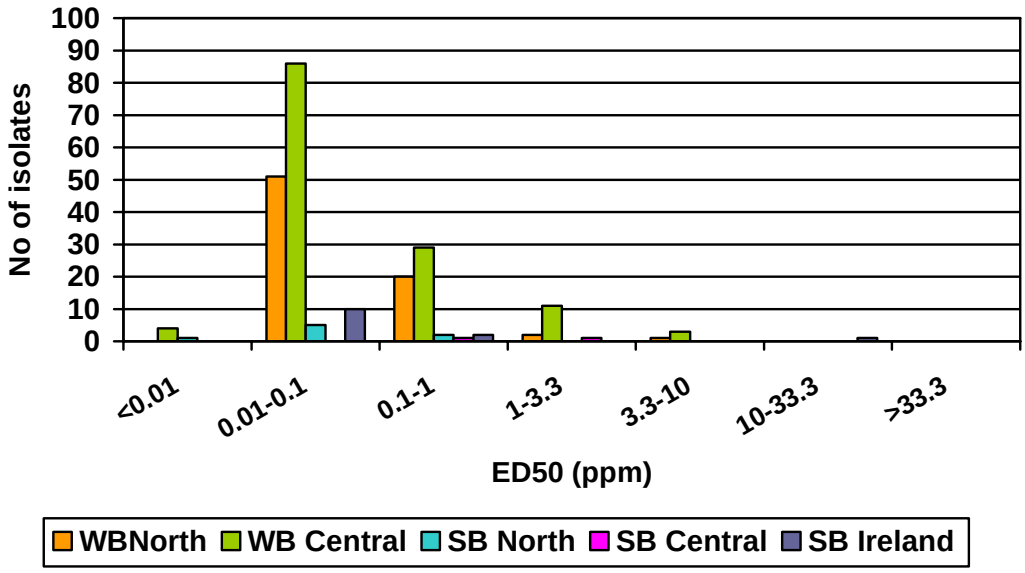

Figure 26. Sensitivity to pyraclostrobin of *R. secalis* isolates from different trial locations.

Isolates were very sensitive to pyraclostrobin at all trials sites with no evidence of any differences in sensitivity distribution between sites (Figure 26).

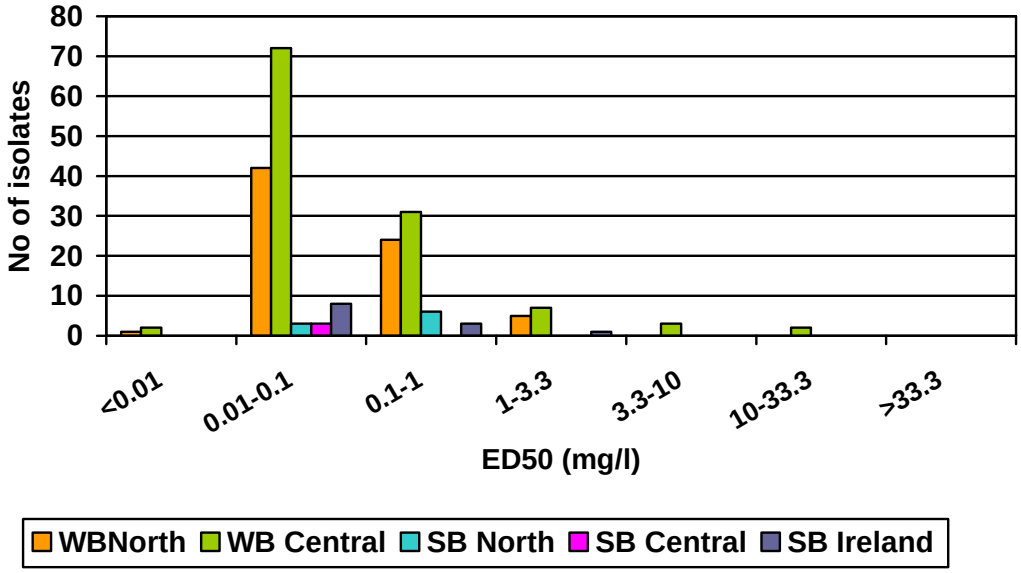

Figure 27. Sensitivity to fluoxastrobin of *R. secalis* isolates from different trial locations.

As with pyraclostrobin, there was no evidence of variations in sensitivity to fluoxastrobin between trial sites (Figure 27). Most isolates had ED_{50} values falling in the most sensitive categories.

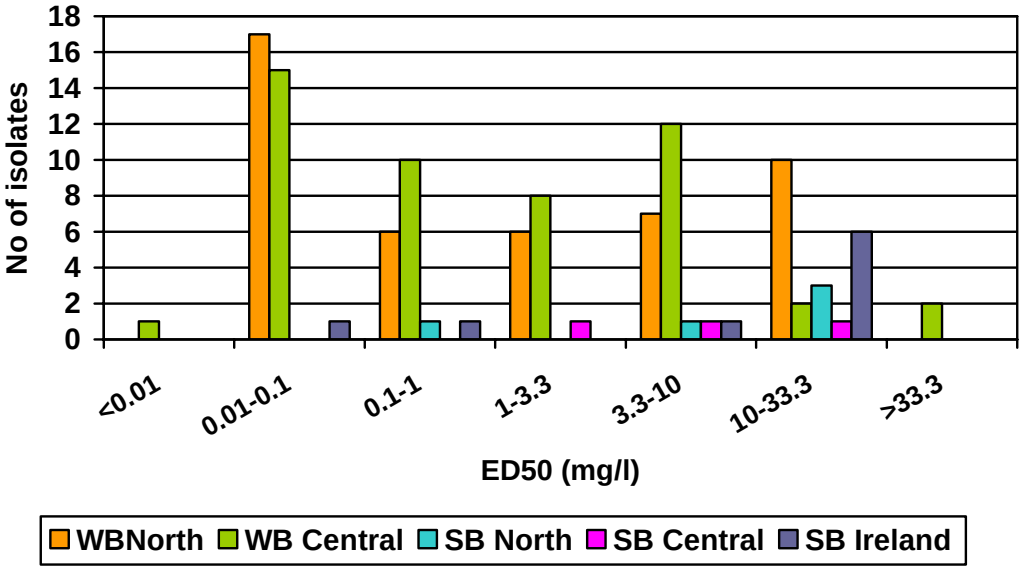

Figure 28. Sensitivity to cyprodinil of *R. secalis* isolates from different trial locations.

There was a range in sensitivity to cyprodinil but overall sample numbers were low so that only trends could be identified. There was a trend for the isolates sampled from Ireland to have ED_{50} values in the least sensitive categories and proportionally fewer in the most sensitive categories (Figure 28). There were no differences between isolate sensitivities to cyprodinil from trial sites in Scotland.

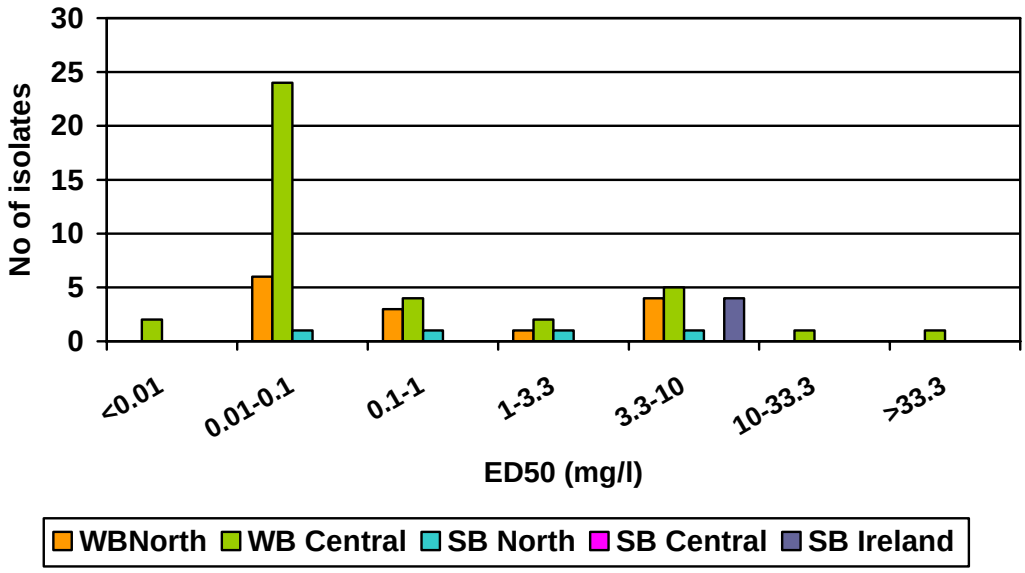

Figure 29. Sensitivity to fenpropimorph of *R. secalis* isolates from different trial locations.

There were significant (P=0.02) differences in the sensitivity of isolates to fenpropimorph that were related to the trial site from which they were sampled, in common with the other SBI fungicides, epoxiconazole and prothioconazole (Figure 29). The isolates from the central Scottish winter barley trial site had ED_{50} values in the more sensitive categories when compared to those from the northern winter barley sites. Sample numbers from spring barley sites were too low for trends to be evident.

Variation by treatment

There were significant differences in sensitivity to epoxiconazole (P=0.02)and to prothioconazole (P<0.001) relative to the sample timing such that isolates sampled early in the season were more likely to have ED_{50} values in the more sensitive categories than those isolates sampled later in the season after treatments had been applied to the trials (Figure 30).

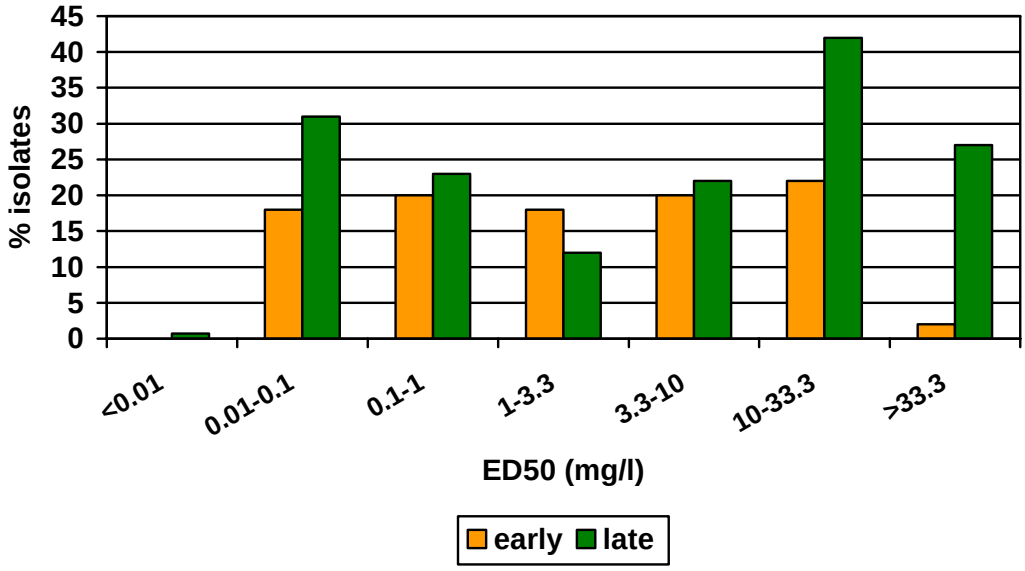

Figure 30. Percentage of *R. secalis* isolates in each sensitivity category for epoxiconazole early and late season.

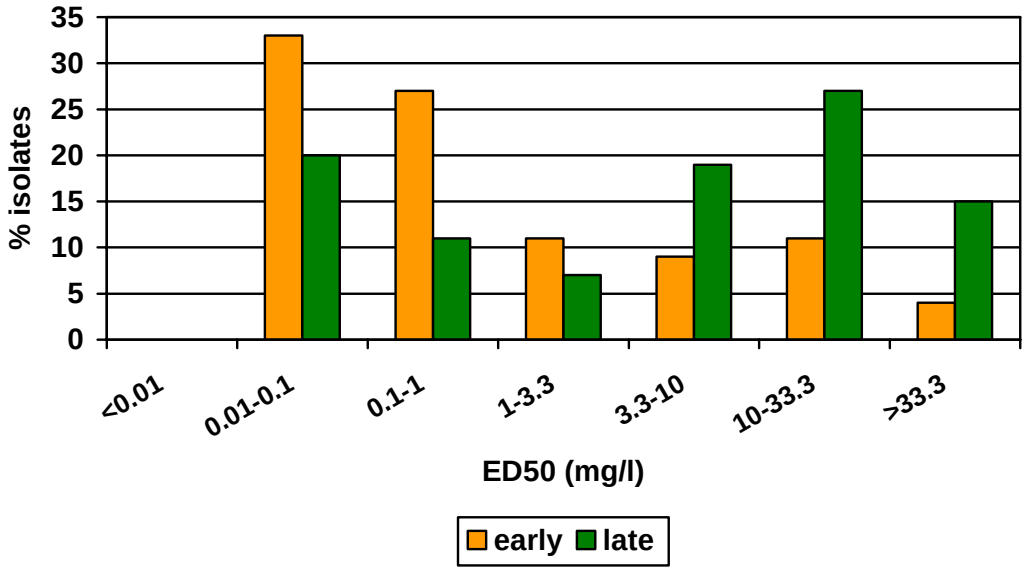

Figure 31. Percentage of *R. secalis* isolates in each sensitivity category for prothioconazole early and late season.

R. secalis sampled later in the season had more isolates with reduced sensitivity to prothioconazole ($P < 0.001$) than those taken early in the season which tended to have more isolates in the sensitive ED_{50} categories (Figure 31).

Significant shifts were also seen for cyprodinil ($P< 0.001$) and fenpropimorph ($P= 0.02$) No early and late differences were noted for the two strobilurin fungicides

(fluoxastrobin and pyraclostrobin) and the above observations can be further explained by examining the sensitivity of isolates pre and post specific treatments, although sample numbers were very reduced in some of the most intensive two and three way mixed treatments which reduces the statistical significance of some apparent trends in the figures below.

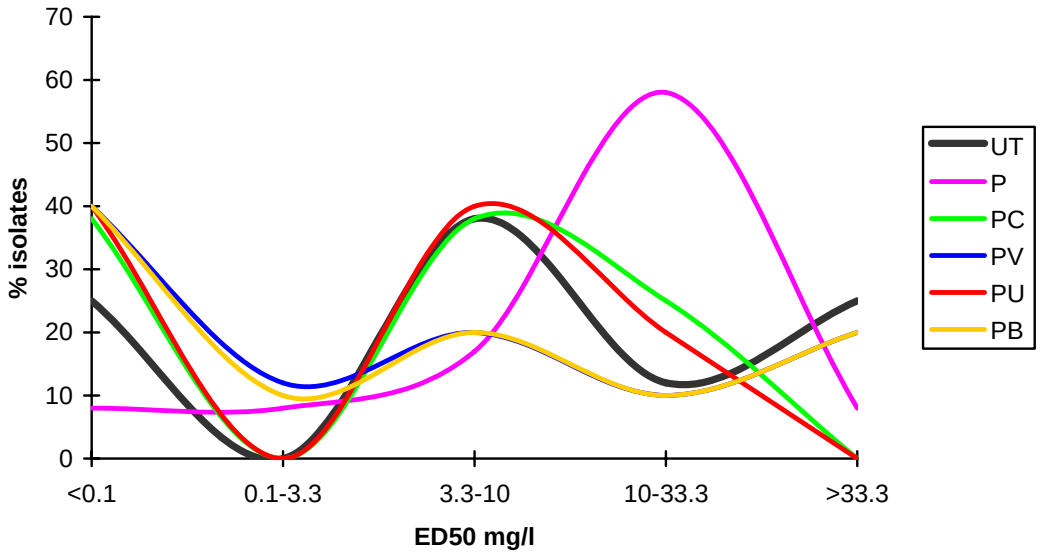

Figure 32. Percentage of *R. secalis* isolates in each sensitivity category following prothioconazole treatment straight or in two-way mixture.

The sensitivity of isolates to prothioconazole had a bimodal distribution (Figure 31). There were more isolates in the higher ED_{50} categories following treatment with straight prothioconazole. This was not the case when the prothioconazole was mixed with either chlorothalonil (B), cyprodinil (U), pyraclostrobin (V), fluoxastrobin (not shown) or fenpropimorph (C). Where prothioconazole was applied in a two or a three-way mixture the sensitivity of isolates was not significantly different from that of the untreated controls.

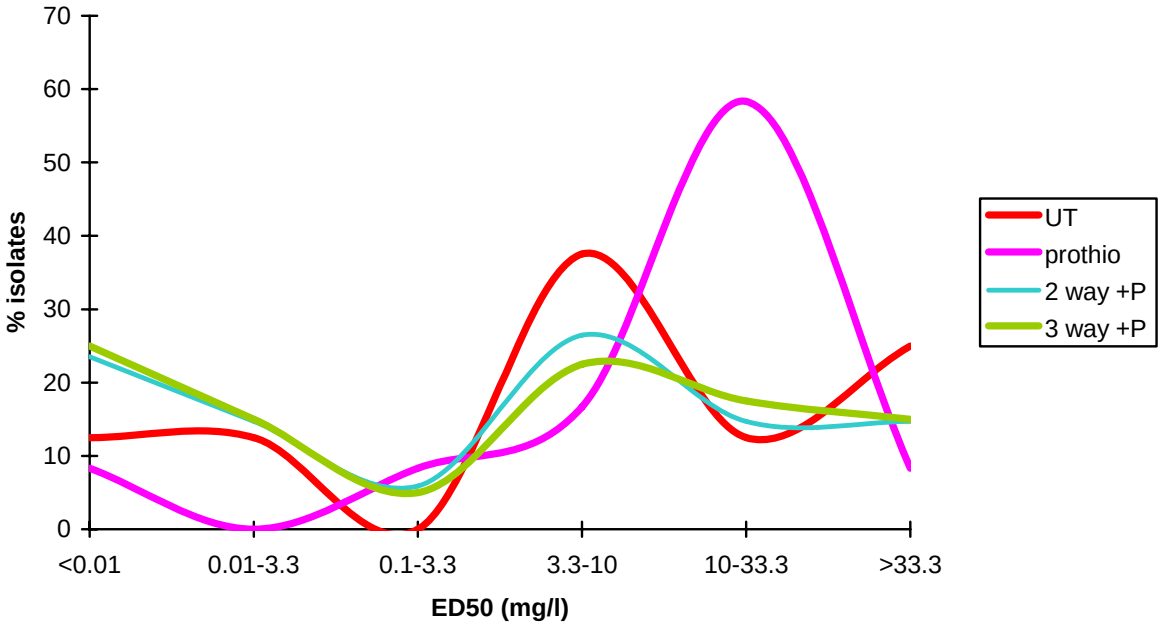

Figure 33. Percentage of isolates in each sensitivity category following prothioconazole treatment straight or in two or three-way mixture.

Numbers of *R. secalis* isolates recovered from three way fungicide treated plots were limited and therefore statistical significance is limited. There was no evidence of any alteration in sensitivity to prothioconazole amongst isolates sampled from three-way mixtures as compared to two-way mixtures or the untreated controls (Figure 33). There was an apparent small shift to reduced sensitivity following prothioconazole + pyraclostrobin + chlorothalonil (PVB) treatment and following prothioconazole + fenpropimorph + cyprodinil (PCV) but sample numbers were very small and this was not statistically significant.

Discussion

There are several key conclusions from the results. The sensitivity to prothioconazole of the *Rhynchosporium secalis* population is highly variable with isolates identified with reduced sensitivity compared to the most sensitive isolates in the population, even though prothioconazole is still highly effective in controlling disease and delivering yield,. There is also strong cross resistance to epoxiconazole, the other DMI evaluated. There have been documented reductions in efficacy associated with epoxiconazole use (Cooke *et al*., 2004) and the data, whilst reassuring in that there was no discernable shift in sensitivity to prothioconazole in the *Rhynchosporium secalis* population over the duration of the project, highlight the potential risk to this product.

The data from this project confirm previous reports of 'hot spots' of reduced sensitivity to epoxiconazole. The data from different trial sites show that the site in the north-east of Scotland had higher proportions of resistant isolates than were found at any other site. Conversely none of the isolates sampled from the trial sites in Ireland were in the most resistant category, with the range of sensitivities also much smaller. This situation could be short-lived if disease is spread by movement on seed.

There was evidence of a shift in sensitivity to prothioconazole following its application as a single active ingredient treatment in the field trials, but this shift was not evident where the fungicide was applied in mixtures. This result supports the use of mixtures as a strategy to reduce the risk of resistance arising in the *R. secalis* population. There was no evidence of any additional benefit from the use of three-way mixtures and no further reduction in the trend towards resistance was observed.

Overall Conclusions

Using a single fungicide will not achieve the most cost-effective disease control in winter or spring barley. At the application time of GS25-32, increasing the number of fungicides in a mixture increases yield in winter and spring barley with no mixture having a detrimental effect on yield. The most cost effective approach at £175/tonne grain prices is to use a three component mixture in winter barley and a two component mixture in spring barley. At reduced grain prices, a fungicide mixture comprising two components is the most cost effective.

Prothioconazole was a key component of fungicide mixtures in winter and spring barley in order to achieve effective disease control, yield and margin. Activity still remained acceptable in field trials where DMI resistant rhynchosporium was present. Resistance is increased to this fungicide where it is applied alone. This practice must therefore be actively discouraged. There is also cross-resistance between the different DMIs. Should triazoles be affected by EU legislation or a major shift in fungicide resistance to this group occurs, growers would have to rely upon cyprodinil and a strobilurin fungicide as the core mixture components. Loss of a second fungicide group due to resistance would lead to major challenges in managing rhynchosporium in winter barley.

Chlorothalonil provides strengths in disease protection. However, where eradicant activity against rhynchosporium is required, chlorothalonil can be detrimental to a mixture comprising two components except in mixture with prothioconazole. This effect is minimal in a three component mixture. Fenpropimorph provides minimal control of rhynchosporium except in a three component mixture in situations where disease eradication is required. The strobilurin pyraclostrobin has key strengths where eradication of rhynchosporium is required, but used alone is weak. Cyprodinil achieves best yields in winter barley and should be a major component of a mixture in “triazole resistant hotspots”. If the hotspots increase, there will be greater reliance on this fungicide in the future to manage rhynchosporium.

Using diagnostics immediately before treatment is a good predictor of potential disease risk and may have advantages over a visual assessment, particularly in spring barley where symptoms at time of treatment are rare. Weather conditions in the spring will play a key part in the potential epidemic, however. The results from the late testing of leaves for rhynchosporium show yield responses to fungicide are higher

where rhynchosporium DNA levels are high, even in the absence of symptom development. This has implications to disease management and also to plant breeders, since varietal resistance to symptoms alone does not negate the need for fungicides to achieve the highest yield. Diagnostics are likely to be a useful tool for breeders to determine varieties with true resistance where growth of the pathogen is suppressed as well as symptom development.

References

Babij, J., Zhu, Q., Brain, P. & Hollomon, D.W. (2000). Resistance risk assessment of cereal eyespot, *Tapesia yallundae* and *Tapesia acuformis*, to the anilinopyrimidine fungicide, cyprodinil. European Journal of Plant Pathology 106, 895-905.

Clark, W.S. (1992). Practical aspects of resistance to DMI fungicide in barley powdery mildew *Erysiphe graminis*. Brighton Crop Protection Conference – Pests and Diseases 1, 177-182.

Cooke, L.R. & Locke, T. (2002) Fungicide programmes for effective control of Rhynchosporium on barley. HGCA Project Report no. 290. pp32.

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. (2004). The effect of programmes based on epoxiconazole on the control and DMI sensitivity of *Rhynchosporium secalis* in winter barley. Crop Protection, 23, 393-406.

Fountaine JM, Shaw MW, Napier B, Ward E & Fraaije BA (2007) Application of real-time and multiplex PCR assays to study leaf blotch epidemics in barley. *Phytopathology* 97, 297-303.

Fraaije, B. A., Cools, H. J., Fountaine. J. M., Lovell, D. J., Motteram, J., West, J. S., and Lucas, J. A. 2005. QoI resistant isolates of Mycosphaerella graminicola and the role of ascospores in further spread of resistant alleles in field populations. Phytopathology 95:933-941.

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

Heaney, S.P., Hall, A.A., Davies, S.A. & Olaya, G. (2000). Resistance to fungicides in the QoI-STAR cross-resistance group: current perspectives. The BCPC Conference – Pests and Diseases 2, 755-762.

Heaney, S.P., Hutt, R.T. & Miles, V.G. (1986). Sensitivity to fungicide of barley powdery mildew populations in England and Scotland. 1986 British Crop Protection Conference – Pests and Diseases 2, 793-800.

Hollomon, D.W. (1997). 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. HGCA Project Report No. 139, 36 pp.

Hollomon, D.W., Cooke, L.R. & Locke, T. (2002). Maintaining the effectiveness of DMI fungicides in cereal disease control. HGCA Project Report No. 275, 24 pp.

Gisi, U., Sierotzki, H., Cook, A. & McCaffery, A. (2002). Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Management Science 58, 859-867.

Kendall, S.J., Hollomon, D.W., Cooke, L.R. & Jones D.R. (1993). Changes in sensitivity to DMI fungicides in *Rhynchosporium secalis*. Crop Protection 12, 357-362.

Napier, B.A.S., Bayles, R.A., Stigwood, P.L. & Burnett, F.J. (2000). Sensitivity of powdery mildew and yellow rust to DMI, morpholine and strobilurin fungicides in England and Scotland. The BCPC Conference – Pests and Diseases 1, 427-434.

Taggart, P.J., Cooke, L.R., Mercer, P.C. & Shaw, M.W. (1998). Effects of fungicides used to control *Rhynchosporium secalis* where benzimidazole resistance is present. Crop Protection 17, 727-734.

Taggart, P.J., Locke, T., Phillips, A.N., Pask, N., Hollomon, D.W., Kendall, S.J., Cooke, L.R. & Mercer, P.C. (1999). Benzimidazole resistance in *Rhynchosporium secalis* and its effect on barley leaf blotch control in the UK. Crop Protection, 18, 239-243.

Wolfe, M.S. (1984). Trying to understand and control powdery mildew. Plant Pathology 33, 451-466.

Zhang, J., and Stewart, J. M. 2000. Economical and rapid method for extracting cotton genomic DNA. J. Cotton Sci. 4:193-201.