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MONITORING AND FORECASTING FUNGICIDE RESISTANCE IN *RHYNCHOSPORIUM*

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MONITORING AND FORECASTING FUNGICIDE RESISTANCE IN RHYNCHOSPORIUM

by

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Final report of a three year project co-ordinated by Dr. D. W. Hollomon, IACR-Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, and involving several research collaborators. The work commenced in May 1987 and was funded by a grant of £72,352 from the Home-Grown Cereals Authority (Project No. 0011/3/87).

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INTRODUCTION

Leaf blotch (*Rhynchosporium secalis* (Oudem) J.J. Davis) is a damaging disease of barley in cool, maritime climates, and serious losses can occur when epidemics coincide with grain filling (Jenkins and Jemmett, 1967). Although resistant cultivars contribute to leaf blotch control (Anon, 1991), autumn sown cultivars generally lack effective host-plant resistance, and fungicides offer the only effective means of control (Martin and Morris, 1979). These same fungicides are used against other barley diseases, so that even when leaf blotch is not important, *R. secalis* populations are subjected to some selection for fungicide resistant strains. The extent of variation in 'base-line' sensitivity to the triazole fungicide, triadimenol, and on which selection may act, was revealed by a survey of 30 *R.secalis* strains collected from field crops between 1975 and 1981. At least a 10-fold range in sensitivity was detected, although even the least sensitive strains were adequately controlled on treated seedlings (Hollomon, 1984). Limited surveys carried out in 1985-6 involving 90 isolates collected from S.W. England and Scotland, suggested that the mean sensitivity of *R. secalis* isolates to triadimenol had, by then, declined 5-10 fold (Hollomon, unpublished data). The present work extended these surveys to all parts of the UK, and included measurements of sensitivity to two other DMI fungicides, propiconazole and prochloraz. Performance changes were monitored in a series of field experiments, which were also used to evaluate strategies of fungicide use to combat the development of resistance to DMI fungicides.

Carbendazim, and other MBC-generating fungicides are also important for Rhynchosporium control, especially in mixtures with triazole and other DMI fungicides. These mixtures are also used for control of *Pseudocercosporella herpotrichoides* (eyespot). Until 1988, none of the 4,000 strains tested grew on media containing 1 μg/ml carbendazim, despite the fact that mutants able to grow at this carbendazim concentration were readily generated in the laboratory. In 1989, however, one isolate from N. Ireland was carbendazim-resistant, although it grew poorly in culture and was eventually lost. In this report we identify further field strains that are carbendazim resistant, grow normally in culture, and are pathogenic.

METHODS

Techniques for the isolation and assay of *R. secalis* have been described elsewhere (Hollomon, 1984; Jones, 1990). At least 10 field trials were conducted in different parts of the UK during the course of this work. Fungicide treatments varied depending on local advisory needs, but if necessary were applied on two occasions at GS31-33 and 57-59. The percentage leaf area infected by *R. secalis* was assessed on three fully expanded leaves of 10 randomly selected tillers, using the key described by James (1971). Assessments were made five days before each fungicide treatment, and 30 days after the second (GS 80-85). Crops were sprayed with Calirus (benodanil) for brown rust control if necessary.

RESULTS

Changes in sensitivity to DMI fungicides

a. **Triadimenol**

2,074 single spore isolates were assayed for triadimenol sensitivity during the four years of the project. Isolates were obtained from throughout the UK, from both fungicide treated and untreated sites. Similar sites were sampled each year except in 1989, when extreme hot weather limited Rhynchosporium to winter barley crops in S.W. England. Mean sensitivity to triadimenol of the population sampled declined during this period, and by 1990 was 43-fold less sensitive when compared with results from an albeit smaller survey carried out in 1981 (Figure 1). A distinctly bimodal population distribution was observed in 1987, but by 1990 the wild-type population had largely been replaced by the less sensitive one.

These surveys failed to show significant regional differences in triadimenol sensitivity, even though in areas such as N. Ireland fungicide use was much less intensive than elsewhere. Isolates from treated and untreated crops were not significantly different although sample sizes from treated areas were often too small to allow statistically meaningful comparisons. There was no correlation between triadimenol sensitivity and virulence characteristics.

b. **Propiconazole**

From 1986 onwards nearly 2,000 isolates were assayed for propiconazole sensitivity. Mean sensitivity declined nearly eight-fold by 1990 (Figure 2), but quite unlike for triadimenol, change involved a shift in a unimodal distribution, with considerable overlap of the populations occurring throughout. There was no correlation between propiconazole use and sensitivity, and the pattern of change was the same throughout the UK.

c. **Carbendazim**

Prior to 1988, routine tests on all strains failed to detect any which grew on media containing 1 μg/ml carbendazim, a concentration widely used as a discriminating dose to identify resistance. The first strain which grew at this carbendazim concentration was isolated in N. Ireland in 1988, but it grew poorly in culture and was lost. The following year four carbendazim-resistant strains were obtained, and all were easily maintained in culture. In 1990, 1% of the total population sampled were resistant, and some of these strains were able to grow in the presence of 50 μg/ml carbendazim.

Cross-resistance patterns

Analysis of MIC data obtained for 48 strains from one field site in 1989 using Spearmans rank correlation showed that cross-resistance existed between triadimenol and propiconazole, but not to prochloraz (Table 1). This result reflected the pattern observed in the more extensive UK survey, where data were available for all three fungicides for 1700 strains. Indeed, when comparisons were confined to strains with an MIC value for propiconazole of 5 μg/ml or above, the correlation coefficient for propiconazole and triadimenol approached 1, with all but one of these strains able to grow on media containing 25.6 μg/ml triadimenol or more.

Pathogenicity

Infection of barley seedlings with some *R. secalis* strains resistant to triadimenol or carbendazim produced smaller and fewer lesions than infection caused by wild-type sensitive strains (Table 2). However, for other resistant strains infection patterns on seedlings were identical with those of wild-type strains. Despite differences in pathogenicity, control of resistant strains by either triadimenol (Baytan) or carbendazim (Bavistin) was reduced when compared with that of wild-type sensitive ones.

Effects on field performance

To show that changes in sensitivity to DMI fungicides observed in laboratory tests have affected performance, good, reliable field data are needed preferably from sites where Rhynchosporium populations have not been exposed to azole fungicides. Initial control of Rhynchosporium with DMI fungicides was good (Martin and Morris, 1979), and two trials carried out by the collaborators prior to the start of this HGCA project confirmed this. One trial carried out in Devon in 1983, using a natural field population of Rhynchosporium, which at that stage was probably sensitive to DMI's, gave over 90% control from a single Bayleton (triadimefon) spray (Jones, 1990; Table 3). In a trial at Aberystwyth involving artificial inoculation with Rhynchosporium isolates never exposed to fungicides, control was again good with either Bayfidan (triadimenol) or Tilt (propiconazole).

By contrast, in all field trials conducted during this HGCA programme, even two sprays of Bayfidan gave poor results. At one Scottish site, where the mean MIC value for triadimenol from 74 Rhynchosporium isolates obtained from the trial was 44.5 μg/ml, Tilt was little better (Table 4). Sportak provided the best control on this occasion.

At Hillsboro in N. Ireland, where 75% of the Rhynchosporium isolates had a MIC value for triadimenol of 12.8 μg/ml or above, a single well timed spray of Tilt gave no more than 50% control of the disease at GS 51, one month after application. Overall, at this site Hispor (propiconazole + MBC) gave the best result.

In three field trials in S.W. England in 1988, and a further 2 in 1989, control following a single spray of Bayfidan at GS 31, never exceeded 50% (Table 5). A single application of Tilt was little better, although a second spray with this fungicide at GS 39 gave acceptable control of Rhynchosporium. At all these sites the Rhynchosporium population was more sensitive to DMIs than was the population at the Scottish and N. Ireland locations.

A final field trial was carried out at Long Ashton in 1990 using the susceptible cultivar Doublet grown as an autumn sown crop. The mean MIC for triadimenol of the Rhynchosporium population at this site was 42.3 μg/ml. No significant control of Rhynchosporium was achieved following two sprays of Bayfidan (Table 6). Radar (propiconazole), Sportak (prochloraz) and Hispor all performed better. Extensive monitoring of isolates recovered from different plots at this site showed that whereas propiconazole exerted some selection pressure, and overall sensitivity declined in Radar treated plots, triadimenol (Bayfidan) no longer affected sensitivity (Figure 3). Prochloraz was the most effective DMI fungicide in this trial and, reflecting the lack of cross-resistance, apparently exerted no selection pressure on the Rhynchosporium population for altered DMI sensitivity.

DISCUSSION

The field experiments carried out during this HGCA programme contrast with earlier ones, and with experiments where plots were inoculated with wild-type *R. secalis* strains. Comparisons between these experiments are limited by differences between cultivars, infection levels at the time of spraying, and weather conditions, but the performance of triadimenol (and triadimefon) appears to have declined since it was introduced some 10 years ago. No correlation between triadimenol sensitivity and fungicide treatment was observed within each experiment, suggesting that Rhynchosporium is more mobile than might be expected for a splash dispersed pathogen. However, differences in sensitivity between field sites shows some correlation with performance (Figure 4).

Although propiconazole is more active against *R. secalis* than triadimenol, shifts in propiconazole sensitivity have been as marked as to triadimenol, and extend beyond the range of the wild-type population. These changes also seem unrelated to disease pressure and have occurred throughout the UK. The population has remained unimodal, and no evidence has emerged of two classes within the population, either from our own surveys, or from data of others (Margot *et al.*, 1990). These changes are quite different from those observed with triadimenol, where a distinct, non-overlapping, resistant population has emerged following selection. This may reflect a difference in the genetic control of the variation in sensitivity to these two fungicides, despite a common mode of action, and suggests a fundamental difference in the selection process. Around 90% leaf blotch control was achieved at some Scottish sites (Kirk and Leadbeater, 1990; Margot *et al.*, 1990) but elsewhere propiconazole (Radar) has not always been as effective, and in several experiments reported here propiconazole failed to achieve 50% control (Table 4). Mixing carbendazim with propiconazole often gave the best disease control, and appeared to counteract selection for reduced propiconazole sensitivity. Whilst these changes in propiconazole sensitivity may lie within the range of the fungicide's efficacy, and performance of propiconazole against leaf blotch may be adequate in many situations, further use of the fungicide alone should be avoided.

Prochloraz is perhaps the most active DMI fungicide examined so far against *R. secalis*. Cross-resistance between it and triadimenol and propiconazole was not marked, and changes in prochloraz sensitivity small. Nevertheless, the performance of Sportak against Rhynchosporium was often erratic, probably on account of its poor systemic action and the need for rainfall to redistribute it across leaf and stem surfaces.

Carbendazim resistance has already developed in several plant pathogens and, despite earlier optimism, pathogenic MBC-resistant strains have now been isolated from field populations of *R. secalis*. Although these resistant strains varied in their pathogenicity they seem likely to survive and spread, especially where carbendazim was used as a single product. Mixing carbendazim with DMI fungicides may delay the spread of carbendazim resistance, but growers should consider diversifying to include DMI/tridemorph mixtures. These generally perform well (Margot *et al.*, 1990; Hollomon *et al.*, unpublished observations) against Rhynchosporium, despite the poor activity of tridemorph alone.

CONCLUSIONS

This project coincided with a period when significant changes were taking place in the sensitivity of *R. secalis* to some DMI fungicides. These changes have been charted through National surveys, and have emphasised the value of early 'baseline' data in confirming shifts in sensitivity. The following points have emerged:

1) A decline in sensitivity has occurred to triadimenol and propiconazole but not to prochloraz. Some limited *in vitro* testing suggests sensitivity to other triazoles has also declined.

2) Change has occurred throughout the UK, irrespective of disease pressure; only remote barley growing areas have escaped. Similar changes have occurred in Germany (Schulz, personal communication, 1989).

3) Selection with triadimenol has generated a bimodal population distribution suggesting that the wild-type sensitive population is being replaced by a less sensitive one. Changes in sensitivity to propiconazole have followed the more usual pattern for DMI fungicides, with a gradual shift of a unimodal population towards a less sensitive mean.

4) Comparisons with early field trials data clearly indicate that the performance of triadimefon/triadimenol (Bayleton, Bayfidan, Baytan) has declined, although this has been difficult to establish convincingly within a single field experiment. Changes in the performance of Tilt/Radar have been less obvious, and the greater activity of propiconazole than triadimenol against *R. secalis* seems to ensure sufficient efficacy remains. However, propiconazole exerted strong selection during 1990, and its use alone should not be encouraged. Performance of Sportak seems largely unchanged, but other DMI fungicides (Impact, Punch) have not been examined in sufficient detail to establish what if any changes have occurred to these widely-used fungicides.

5) Carbendazim-resistant strains of *R. secalis* amounted to 1% of total isolated. All were pathogenic and stable, which illustrates the need to continually monitor pathogen populations in some way. Carbendazim/DMI mixtures still performed well, but diversification to include DMI/tridemorph mixtures for Rhynchosporium seems prudent.

FUTURE RESEARCH

There is clearly a continuing need to monitor carbendazim sensitivity. If practical, DMI/morpholine mixtures should be assessed for Rhynchosporium control. The new triazole, cyproconazole, is now registered for use in the UK, and tebuconazole is likely to follow shortly, and these should allow improved Rhynchosporium control.

ABSTRACT

Resistance has significantly reduced the performance of Bayleton, Bayfidan and Baytan against Rhynchosporium. This has probably occurred throughout most of the UK. A decline in sensitivity to propiconazole has also taken place, but Tilt/Radar still provide worthwhile Rhynchosporium control. However, Tilt exerted strong selection pressure again in 1990, and its use as a single product for Rhynchosporium control is not recommended. Changes in prochloraz sensitivity were small, and the performance of Sportak seems unchanged.

DMI/carbendazim mixtures continue to form the basis for combating any further development of DMI resistance, and these mixtures performed well. However, in 1990, well characterised carbendazim-resistant strains accounted for 1% of the total number of isolates examined suggesting that DMI/morpholine mixtures also need to be exploited for Rhynchosporium control.

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Figure 1

Changes in Triadimenol sensitivity in *Rhynchosporium secalis*

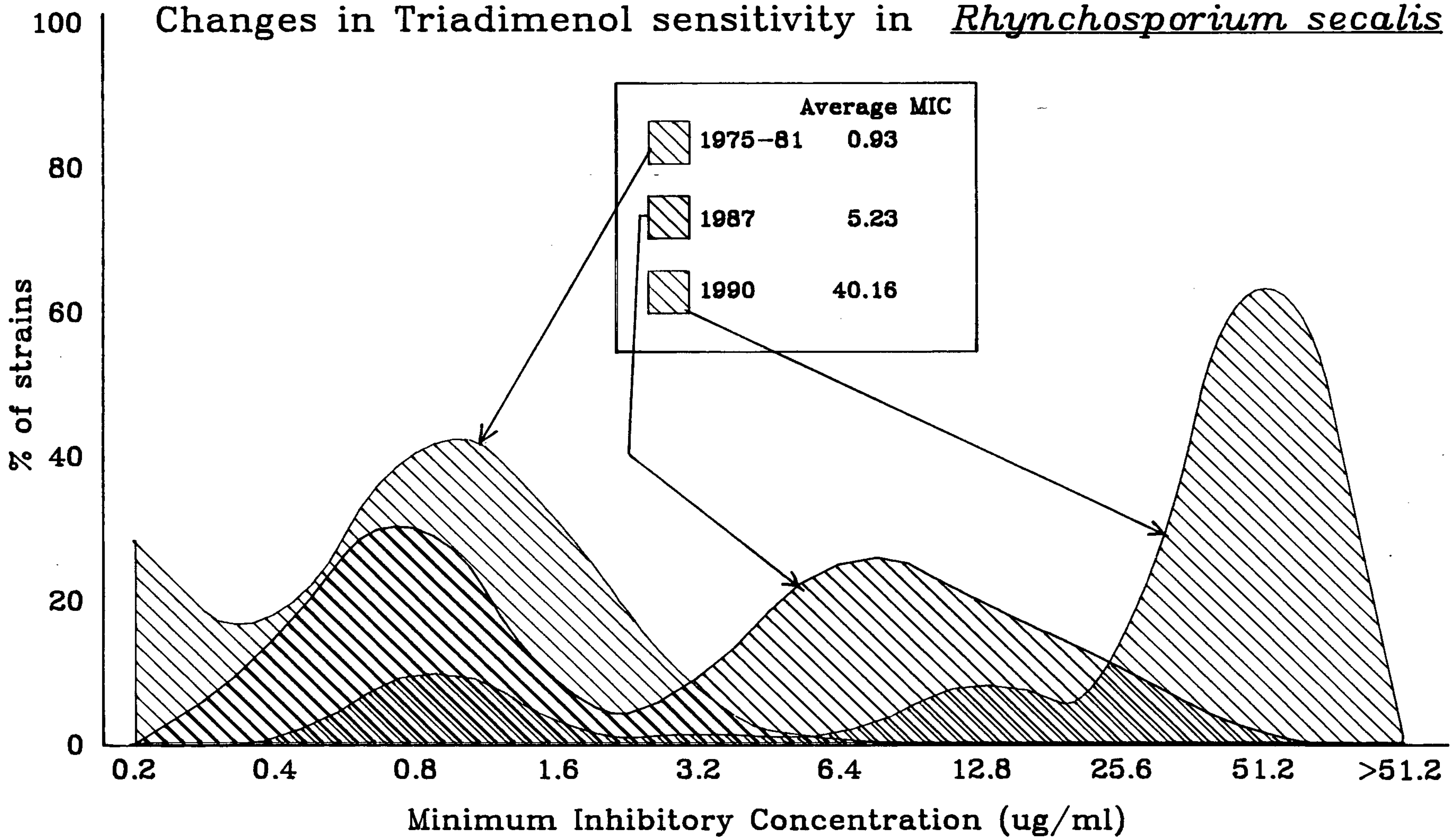

Figure 2

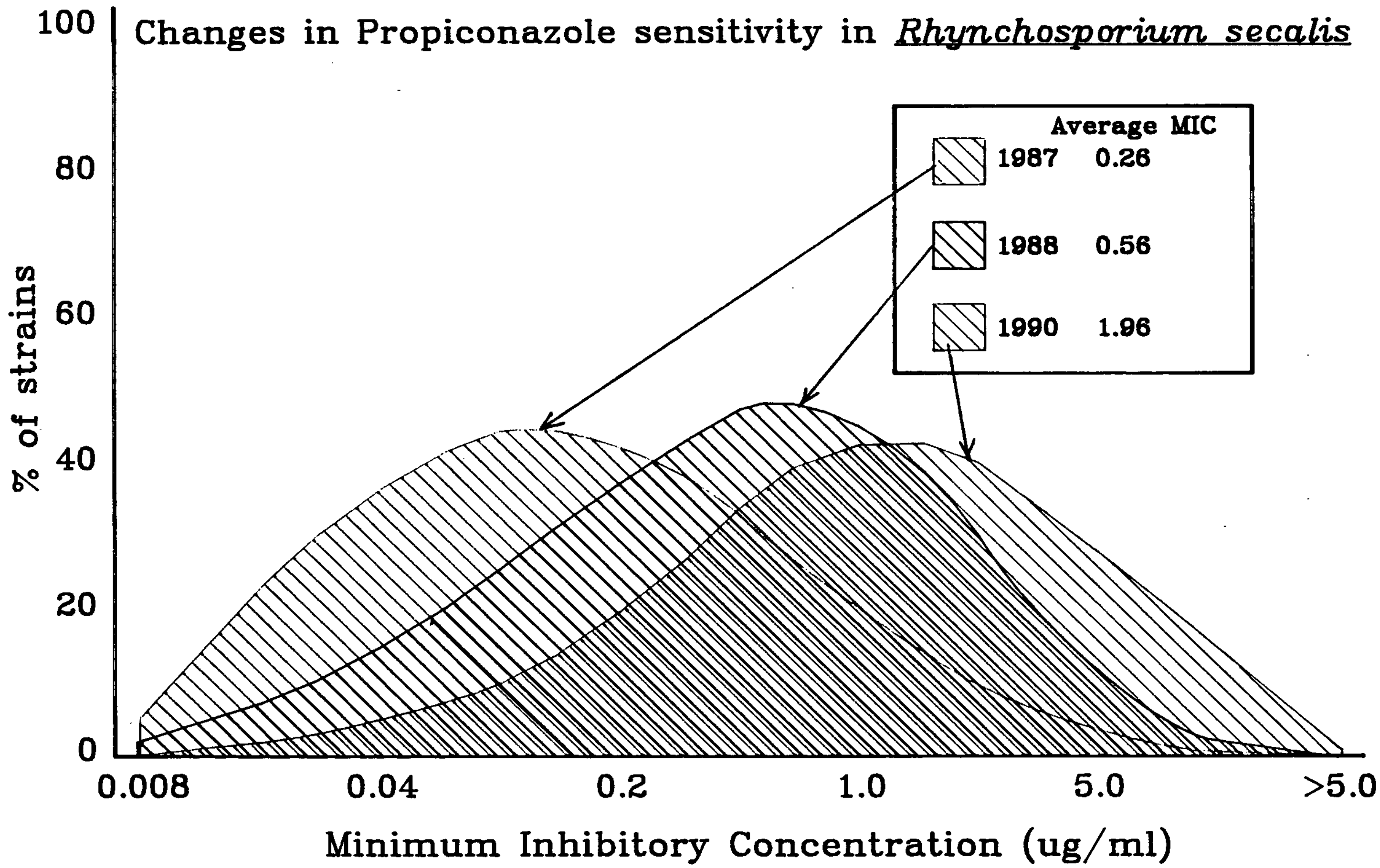
Changes in Propiconazole sensitivity in *Rhynchosporium secalis*
100
80
60
40
20
0
% of strains
Average MIC
1987 0.26
1988 0.56
1990 1.96
0.008
0.04
0.2
1.0
5.0
>5.0
Minimum Inhibitory Concentration (ug/ml)

Figure 3. Sensitivity changes in *Rhynchosporium secalis* from one field site in response to fungicide treatments.

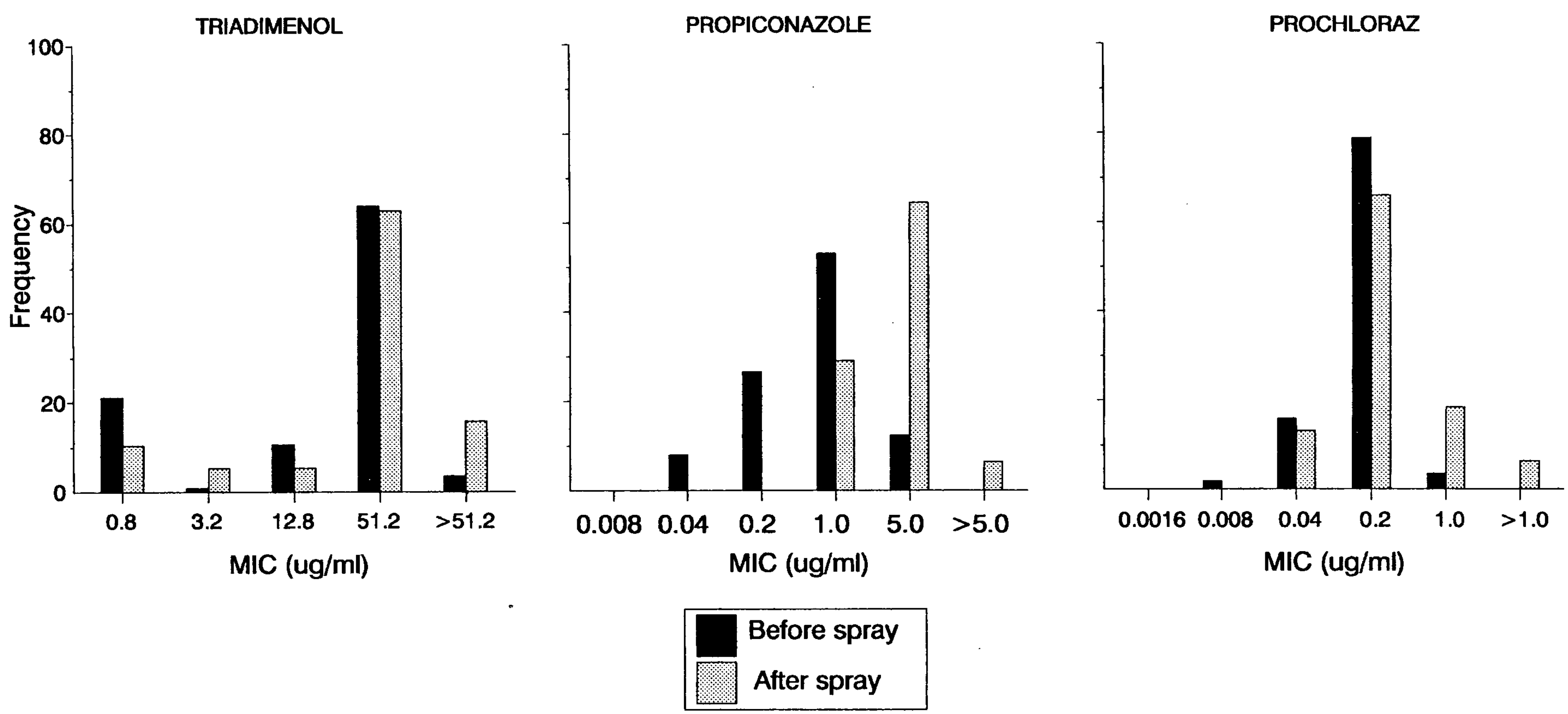

TABLE 1. Cross-resistance patterns in *R. secalis*.

	Triadimenol	Propiconazole	Prochloraz	Carbendazim
Triadimenol				
Propiconazole	0.64**			
Prochloraz	0.11 ns	0.17 ns		
Carbendazim	0.04 ns	0.06 ns	0.12 ns	

Data are correlation coefficients of indexed MIC values

** = Significant at 1%

ns = Not significant.

TABLE 2. Control of *R. secalis* by carbendazim.

	% leaf area diseased			
Carbendazim applied (mg/pot)	Isolate 554.24	672.09 6-1	672.10 2-2	666.02 2-2
None	51.9	67.4	90.5	16.5
50	NT	73.5	84.0	7.8
25	NT	67.1	92.9	6.7
12.5	4.5	76.1	89.0	6.1
6.25	45	67.9	82.7	8.6
3.12	40	65.9	85.4	14.2
LSD 5% between treatments	36	18	23	12

Seedlings (cv. Doublet) inoculated at second leaf stage by spraying to run off with spore suspensions (2.5 x 10^5 spores ml^{-1}).

Seedlings incubated in a dew chamber for 72 h at 16°, and then maintained at 99% RH at the same temperature in a growth chamber for up to 14 days.

Disease assessments made using standard keys.

NT = Not tested.

TABLE 3. Control of Rhynchosporium at two DMI sensitive sites.

Fungicide	% Control	
	Devon 1983	Aberystwyth 1987
Untreated	(15)*	(46)
Bayleton (0.5 kg/ha)	93	-
Bayfidan (0.5 l/ha)	-	87
Tilt (0.5 l/ha)	93	88
Mean triadimenol sensitivity (MIC μg/ml)	ND	0.2

* % leaf area infected at GS 59-75

ND = Not determined

TABLE 4. Control of Rhynchosporium at Newburgh, Grampian and at Hillsboro, N. Ireland in 1988.

	Newburgh % leaf area infected (leaf 2)	Hillsboro % leaf area infected (angular transformation: leaf 2)
	GS 71	GS 51
Untreated	42	17
Tilt	31	9
Bayfidan	45	-
Hispor	31	7
Sportak	19	-
SED (15 df)	10	
Mean triadimenol sensitivity (MIC; μg/ml)	44.5	12.8

cv. Igri

TABLE 5. **Control of Rhynchosporium in S.W. England.**

	1988 (Mean of 3 trials)		1989 (Mean of 2 trials)	
	% leaf area infected GS 75		% leaf area infected GS 59-75	
	Leaf 1	Leaf 2	Leaf 2	Leaf 3
Untreated	30	40	8	16
Bayfidan	16	27	5	7
Tilt	12	20	2	5
Tilt x 2	3	8	1	2
Sportak	19	25	3	4
Hispor	7	14	3	5
Tilt-Turbo	8	15	2	4
SED	2.2	3.7	0.7	0.9
Df	81	81	60	60
Mean Triadimenol sensitivity (MIC = μg/ml)	3.2		1.4	

TABLE 6. **Effect of fungicide treatments on leaf blotch severity and yield.**

Treatment	Leaf blotch severity %*	Disease control %	Yield (tonnes/ha)**
None	54.0 d***	-	5.38
Bayfidan	44.0 cd	18.6	5.49
Sportak	25.3 ab	53.2	5.73
Radar	30.4 bc	43.8	5.56
Hispor	16.8 a	68.9	5.74

* Mean of three leaves at GS 80-85

** To 85% dry matter

*** Treatment means followed by a common letter are not significantly different (p = 0.05, ANOVA).

Figure 4. Triadimenol sensitivity and Rhynchosporium control at several sites throughout UK.