

PROJECT REPORT No. 140

SENSITIVITY OF EYESPOT TO PROCHLORAZ IN ENGLAND IN 1996

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by

D. R. JONES

ADAS Rosemaund, Preston Wynne, Hereford HR1 3PG

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SUMMARY

A survey was undertaken in 1996 to investigate sensitivity to prochloraz of UK populations of the cereal eyespot pathogen, *Pseudocercosporella herpotrichoides*. Sixty-three wheat and barley samples were received and, from over 434 eyespot lesions found, isolation of the pathogen was attempted from 278 lesions. Most attempted isolations were contaminated by *Fusarium* spp., so only 18 isolates of *P. herpotrichoides* were obtained for testing, from 3 fields in south-western England. All were of the Rye-pathotype. There was some variation in ED_{50} s for prochloraz, but every isolate had an ED_{50} below 0.50 mg/l, which is within the range previously found in the UK. ED_{50} s were also determined for flusilazole and propiconazole. There was a much stronger correlation between sensitivity to flusilazole and to propiconazole than between either of these fungicides and prochloraz.

Due to the very limited number of pathogen isolates available for testing, no conclusions can be drawn regarding the sensitivity of the UK eyespot pathogen population to prochloraz.

INTRODUCTION

Eyespot still presents UK farmers with one of the most difficult crop protection decisions, due to the difficulty in assessing the risk that eyespot lesions will become severe and thereby increase the risk of lodging and reduce yield and quality. ADAS/CSL winter wheat disease surveys over the past ten years, on randomly-selected commercial crops of which approximately 95% received at least one fungicide, showed that, in most years, eyespot caused a greater financial loss than any other disease except for Septoria tritici (R W Polley, unpublished). In 1995, the estimated losses from eyespot were £13.4m, greater than the aggregated losses from all foliar diseases (£10.4m). This shows the difficulty in achieving commercially-acceptable control of eyespot in comparison with other diseases. Since the widespread development of resistance to MBC fungicides in the early 1980s, the azole

fungicides prochloraz and flusilazole and, more recently, epoxiconazole have been the only fungicides available for eyespot control. The efficacy of prochloraz for eyespot control was variable in ADAS fungicide experiments in the 1980s, generally giving a reduction in disease of between 30 and 40%, but in some instances over 60% (Jones, 1994; 1995). In spite of this erratic performance, recent HGCA-funded research (Project 0015/01/91) confirmed that prochloraz is the most effective of the currently available fungicides against eyespot when the disease pressure is greatest, although flusilazole is a satisfactory alternative where the disease is less severe.

Poor control of eyespot by prochloraz, beyond the normal variability in disease control, has been reported in parts of northern France since 1991, and the presence of strains of the eyespot fungus less sensitive to prochloraz has been confirmed (Leroux and Marchegay, 1991; Birchmore *et al.*, 1994). There was no information on whether resistance has developed in the UK, but a possible consequence of the relatively low incidence of severe eyespot in the UK in the early 1990s was that sensitivity may have declined without having been detected in the field.

OBJECTIVE

To test isolates of the eyespot pathogen, *Pseudocercosporella herpotrichoides* from 50 UK wheat and barley crops for sensitivity to prochloraz.

MATERIALS AND METHODS

Sampling

The aim was to collect 50 samples from winter wheat and winter barley crops during late April and early May 1996, from four areas of England, namely south-western England, the West Midlands, East Anglia and north-eastern England. However, this was not possible since eyespot incidence in the spring of 1996 was low and very few of the crops inspected at that time had eyespot lesions. A further attempt to collect isolates was made by checking all barley samples submitted to the ADAS Laboratory in June as part of the HGCA-funded project on fungicide resistance in the barley net blotch pathogen (Locke, 1996), but eyespot incidence was also low in these samples. The third and final collection of eyespot samples was made in July 1996, from crops in the ADAS/CSL Winter Wheat Disease Survey. An additional sample was collected for isolation of the eyespot pathogen from each field where the sample collector observed substantial stem-base browning.

A total of 63 samples were processed, 31 winter wheat and 32 winter barley.

Isolation

For all stems received, stem-bases were examined and eyespot lesions were selected for isolation, up to a maximum of 20 isolations per sample. Isolation was not attempted from lesions which were clearly a combination of eyespot with another stem-base disease (sharp eyespot or fusarium foot rot). The isolation medium was potato dextrose agar (PDA) amended with streptomycin at 5.0 mg/l plus chloramphenicol at 5.0 mg/l. Cultures were maintained by sub-culturing onto the same medium at 10-14 day intervals until tested for sensitivity to fungicides.

Sensitivity testing

The original intention was to test 10 isolates per field on agar amended with prochloraz at two concentrations, namely 0.5 and 2.0 mg/l. In view of the sample numbers obtained being far below the target, it was decided to characterise each of the isolates more thoroughly, to determine the $\rm ED_{50}$ values for prochloraz, flusilazole and propiconazole. The $\rm ED_{50}$ is defined as the concentration of the fungicide which gave a 50% reduction in growth of the pathogen in culture. Concentrations of prochloraz and propiconazole were 0.005, 0.01, 0.05, 0.10, 0.25, 0.50, 1.00 and 2.00 mg/l, and flusilazole concentrations were 0.01, 0.10, 1.00 and 2.00 mg/l. There were two plates of each concentration of each fungicide, and results were expressed as the mean radial growth on the two plates after 14 days.

RESULTS

The total number of eyespot lesions observed on the 63 samples received was 434. Of these, 278 were selected as suitable for pathogen isolation. Others were rejected principally because the stems were clearly affected by other stem-base diseases (sharp eyespot and/or fusarium foot rot) in addition to eyespot. From the lesions which appeared sufficiently clean for isolation, the success rate of the isolations was very low. Many cultures were contaminated by Fusarium spp. and some by Rhizoctonia cerealis (sharp eyespot). These fungi grow faster in culture than the eyespot fungus, P. herpotrichoides, preventing the establishment of clean cultures of P. herpotrichoides. As a result, only 18 cultures of P. herpotrichoides were obtained which could be used for sensitivity testing. These were from three fields in southwestern England, one each in Devon, Dorset and Gloucestershire. All cultures were of the Rye pathotype. ED₅₀s and minimum inhibitory concentration for prochloraz (MIC) for each isolate are given in Table 1. MICs are not presented for flusilazole and propiconazole, since 12 of the 18 isolates showed some growth on the highest concentration of flusilazole tested, and all 18 grew on the highest concentration of propiconazole.

Regression analysis showed that there were significant associations between ED₅₀s for the three fungicides, but the association between flusilazole and propiconazole was much stronger than between either of these fungicides and prochloraz:

Prochloraz v flusilazole $r^2 = 0.277, P = 0.014$ Prochloraz v propiconazole $r^2 = 0.345, P = 0.006$ Flusilazole v propiconazole $r^2 = 0.796, P < 0.001$

Table 1. ED_{50} and MIC for each isolate of *P. herpotrichoides*.

C:+0 P-	MIC	ED	ED.	ED ED
Site &	MIC	ED_{50}	ED_{50}	ED ₅₀
isolate	prochloraz	prochloraz	flusilazole	propiconazole
number*	(mg/l)	(mg/l)	(mg/l)	(mg/l)
A1	1.00	0.30	0.64	1.81
A2	0.25	0.14	0.55	0.45
A3	1.00	0.25	0.37	0.94
B1	< 2.00	0.34	1.03	2.45
B2	0.25	0.21	0.80	1.47
В3	1.00	0.28	0.69	1.58
B4	1.00	0.33	0.71	1.61
B5	2.00	0.36	1.00	2.41
B6	0.50	0.16	0.63	1.50
B7	0.50	0.16	0.69	1.25
B8	1.00	0.30	0.89	2.00
B9	2.00	0.14	0.16	0.50
B10	2.00	0.14	0.45	0.73
·C1	0.50	0.28	0.79	2.50
C2	0.50	0.13	0.63	1.38
C3	0.50	0.10	0.59	1.69
C4	1.00	0.25	1.30	3.19
C5	1.00	0.21	0.53	0.97

*	Sites:
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- A Kingsbridge, Devon
- B Dorchester, Dorset
- C Mangotsfield, Glos

Winter barley Winter wheat

Winter wheat

DISCUSSION

Few conclusions can be drawn from this study, due to the low number of isolates obtained. The success rate for isolation of the eyespot fungus from stem-bases, particularly in late June or July is unpredictable, since the other common stem-base pathogens, Fusarium spp. and Rhizoctonia cerealis, grow much faster in culture than P. herpotrichoides. It is unlikely that P. herpotrichoides could be isolated from a mixed infection. Parry et al. (1994) reported that symptomless infection by Fusarium spp. is common, so it is probable that some of the apparently clean eyespot lesions from which isolation was attempted were already colonised by Fusarium spp. Isolation earlier in the year usually has a higher success rate, hence the intention to collect samples in the spring, but the incidence of eyespot in April and early May was so low that very few samples could be obtained at that time.

Although there was variation in sensitivity to prochloraz, the ED_{50} s for prochloraz were all below 0.50 mg/l, which places these isolates within the normal range of sensitivity found in previous UK work (Birchmore *et al.*, 1994). At sites in France where poor eyespot control had been reported, ED_{50} s were considerably higher, up to 2.0 mg/l. The stronger correlation between flusilazole and propiconazole than between either of these fungicides and prochloraz is consistent with the findings with the net blotch pathogen *Pyrenophora teres* (Locke, 1996). Similarly, in *Rhychosporium secalis*, Kendall *et al.* (1993) found that there was very weak cross resistance between prochloraz and any of propiconazole, triadimenol and tebuconazole; flusilazole was not included in that study.

The very small sample size achieved in the present study precludes any firm conclusion about sensitivity to prochloraz of the UK eyespot pathogen population. A larger sample is required, with both Wheat- and Rye-pathotypes from several areas of the UK, in order to determine whether reduced sensitivity to prochloraz is present in the UK and, if so, to determine its extent.

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