FIELD EVALUATION OF AN IMMUNODIAGNOSTIC ASSAY FOR CEREAL EYESPOT

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ABSTRACT

Visual and immunodiagnostic methods were used to follow eyespot (*Pseudocercosporella herpotrichoides*) development in wheat crops at Long Ashton in 1991. Timing of inoculation, cultivar susceptibility and fungicides were used to attempt to generate plots with different eyespot levels. The immunoassay detected eyespot pre-symptomatically, and subsequently a good correlation was generally observed between visual symptoms and antigen levels. In the resistant cultivar, Rendezvous, a link between final yield and antigen levels was only evident at Growth Stage 85, and no threshold value was established to guide earlier fungicide treatments. Eyespot was more severe on the susceptible cultivar, Pastiche, and good correlations were apparent from GS 32 onwards between antigen levels and final disease severity, and these were always better than correlations based on visual symptoms.

INTRODUCTION

Effective use of fungicides depends on both accurate diagnosis and quantification in order to optimise spray timing. The advent of immunodiagnostic kits (Miller *et al.* 1988; Dewey, 1988; Petersen *et al.* 1990; Smith *et al.* 1990; Cagnieul *et al.* 1992) offers new approaches to detection and measurement of fungal diseases, which may be especially useful where visual diagnosis is difficult, or where pre-symptomatic detection is important. As with threshold criteria based on visual diagnosis, the value of serological tests is dependent on the correlations that can be established between antigenic measurements of disease levels and eventual yield loss. This requires field experiments to be carried out over a number of years during which a range of disease and environmental conditions will be encountered. Although the findings reported in this paper are based on just one year's experiment, it nevertheless describes the approach we are taking to establish an antigen threshold for guiding spray timing to control cereal eyespot (*Pseudocercosporella herpotrichoides* (Fron) Deighton) under UK conditions.

METHODS

Field experiment

A field experiment was carried out during 1990/91 on a site at Long Ashton Research Station where wheat had not been grown in at least the previous fifty years. Winter barley had, however, been grown in 1988-89, and oilseed rape in 1990. A randomised complete block design was used with three replicates and 12 m x 8 m plot sizes. A 1 m

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randomised complete block design was used with three replicates and 12 m x 8 m plot sizes. A 1 m strip around the edge of each plot was not sampled and served to isolate neighbouring plots. Cultivar differences, fungicide treatments, and inoculation timing were all used in an attempt to obtain different eyespot levels between plots. The two cultivars used were Pastiche (S) and Rendezvous (R) which differed in susceptibility to *P. herpotrichoides* (Anon. 1990). The two fungicides were prochloraz (Sportak-45) and flusilazole (Sanction). These were applied at Growth Stage (GS) 33 at recommended rates using a hand-held gas operated sprayer. Two inoculation times were used; one in November 1990, the other in February 1991. Inoculation was carried out according to the method described by Bruehl and Machtmes (1985). Five recently isolated *P. herpotrichoides* strains, all apparently rye pathotypes, were grown on autoclaved oat grains for six weeks at 10-16°C, dried and the different isolates mixed together before broadcasting onto plots at a rate of 400 ml infected grains per plot.

Sampling and visual assessments

Detailed assessments were carried out on five occasions; GS 25 (mid tillering); GS 32 (stem elongation); GS 37-39 (Flag leaf visible); GS 65 (Anthers) and GS 85 (Soft dough). Each time, 30 main shoots including their roots were randomly collected from each plot, avoiding a 1 m strip around the edge. On the first three occasions plants were rinsed with a minimum amount of tap water, whilst subsequent samples were simply shaken to remove soil. Stems were assessed visually for both occurrence and severity of eyespot generating an Eyespot Severity Index (ESI; Scott and Hollins, 1974) which ranged from zero to a maximum of 100.

Immunoassay

Assay procedures followed closely those given in the protocol notes supplied with DuPont diagnostic kits. These development kits differed from those now commercially available, since they incorporated a biotin-avidin step to enhance sensitivity (Smith *et al.* 1990). Each stem base, including leaf sheaths, was trimmed free of roots, cut into 40 mm pieces, frozen, and then either crushed in a polythene bag (GS 25), or macerated in a blender. Preliminary results showed that some additional antigen was released if extracts were allowed to stand before testing. To standardise the procedure extracts were prepared, and then allowed to stand overnight at 4°C before assay the following day.

RESULTS

Effect of cultivar on eyespot development

Eyespot symptoms were not observed on either cultivar before GS 32. Pre-symptomatic detection was possible using immunoassay, but infection and early development were no different in either cultivar. Only subsequently was development and symptom expression reduced on cv. Rendezvous compared to that on cv. Pastiche, with the result that differences were seen between cultivars from GS 37-39 onwards (Table 1). The drop in antigen levels observed at GS 37-39 may reflect a natural loss of infected leaf sheaths.

TABLE 1

Eyespot development on two wheat cultivars.
Results averaged over fungicide treatments and inoculum levels.

	Eyespot levels									
	GS 25		GS 32		GS 37-39		GS 65		GS 85	
	EAU*	ESI+	EAU	ESI	EAU	ESI	EAU	ESI	EAU	ESI
Rendezvous	1274	0	1738	3.4	578	7.1	1581	20.0	4882	40.7
Pastiche	1208	0	1338	8.7	772	18.4	4516	42.8	28,855	67.0
LSD 5%	474		180	3.1	132	3.2	1487	6.6	5434	9.0

* EAU Eyespot Antigen Units
+ ESI Eyespot Severity Index

Effect of fungicides on eyespot development

Assessments were made of sharp eyespot (*Rhizoctonia cerealis*) and Fusarium levels, but eyespot was clearly the major stem-base disease in this trial. Some foliar infection with *Septoria tritici* occurred later in the season. Both prochloraz and flusilazole achieved similar results, and within two weeks of spraying ESA and ESI measurements were always lower in treated than in untreated plots (Table 2). Control of eyespot was, however, only moderate, no doubt reflecting the high disease pressure, and that treatment should have occurred earlier. No interaction was seen between fungicides and either inoculation level or cultivar.

TABLE 2

Effect of fungicides on eyespot development.
Results averaged over cultivar and inoculum levels.

	Eyespot levels								Yield tonnes/ ha**
	GS 32*		GS 38		GS 65		GS 85		
	EAU	ESI	EAU	ESI	EAU	ESI	EAU	ESI	
No fungicide	1382	6.6	1040	18.9	4794	40.7	27,552	69.8	8.49
Prochloraz			495	11.0	1770	27.2	10,515	44.4	9.24
Flusilazole			491	9.4	2582	26.2	12,540	47.3	9.37
LSD 5%			132	3.2	1487	6.6	5,434	9.0	0.15

* Before fungicide application
** At 15% moisture content

Effect of inoculation level on eyespot development

Variation of inoculum timing and level was the most effective way used in this trial to influence eyespot levels. Inoculation increased eyespot severity and antigen levels from GS 32 onwards, especially on cv. Pastiche (Table 3). Eventually EAU levels reached 3-4 times those of uninoculated plots.

TABLE 3

Effect of inoculation timing on eyespot development.
Results averaged over cultivar and fungicide treatment.

	Eyespot levels									
	GS 25		GS 32		GS 37-39		GS 65		GS 85	
	EAU	ESI	EAU	ESI	EAU	ESI	EAU	ESI	EAU	ESI
No inoculation	1250	0	1450	2.1	474	9.2	1666	21.5	8,357	44.5
November 1990	1302	0	1800	10.0	893	16.1	4624	40.4	24,420	63.2
February 1991	1172	0	1546	6.1	614	12.9	3216	32.5	17,698	53.8
LSD 5%	474		180	3.1	132	3.2	1487	6.6	5434	9.0

DISCUSSION

Significant amounts of eyespot antigen were detected in both cultivars from GS 25 onwards, and before visual symptoms appeared. By GS 37-39 differences in both antigen and disease levels had developed because eyespot spread more slowly in cv. Rendezvous than in cv. Pastiche. It seems that initial infection is not reduced in cv. Rendezvous and using these two cultivars to generate different eyespot levels during early epidemic phases was not successful.

Prochloraz and flusilazole provided significant eyespot control on both cultivars, and generated differences between plots in antigen and disease levels. By delaying treatment until GS 33, when antigen levels at GS 32 were known, the opportunity to alter eyespot levels in the early phases of the epidemic was missed. Overall, results were similar to those reported by Bateman (1990) using just visual assessments to assess fungicide performance. Prochloraz and flusilazole performed similarly despite the fact that only rye pathotypes were used to inoculate plots, and in greenhouse trials prochloraz is more active against these pathotypes than is flusilazole (Cavelier *et al.*, 1987).

Inoculation timing produced differences in eyespot levels at GS 32 and later. This was especially so for the susceptible cultivar, Pastiche, although the extent of any differences was limited by the surprisingly high level of antigen (and later disease) in uninoculated plots, despite it being a first wheat crop. This suggests that dispersal may not be restricted to rainsplash and that other, perhaps wind borne, inoculum sources can play a significant part in eyespot epidemics.

TABLE 4

Correlation between eyespot antigen units and either visual disease assessments or yield. Percentage variance accounted for in a regression of eyespot antigen units and yield.

Growth stage	Correlation co-efficients		% variance accounted for	
	Rendezvous	Pastiche	Rendezvous	Pastiche
	Eyespot Antigen Units and Eyespot Severity Index			
32	0.3	0.61**	0	34.2
38	0.38	0.78**	11.3	58.7
65	0.54**	0.87**	25.8	78.0
85	0.78**	0.86**	59.1	80.5
	Eyespot Antigen Units and Yield			
32	0.06	0.04	0	0
37-39	0.19	0.63**	0	38.6
65	0.04	0.70**	0	46.2
85	0.61**	0.61**	36.5	29.6
	Yield and Eyespot Severity Index			
32	0.16	0.12	0	0
37-39	0.32	0.73**	6.9	46.7
65	0.02	0.50**	0	19.0
85	0.58**	0.51**	44.3	21.4

** Significant at 5% probability level.

Despite these limitations, some useful correlations were identified from the data available. There was an increasing correlation between Eyespot Severity Index and antigen units on both cultivars as the season progressed (Table 4). For Pastiche this was significant on all four sampling occasions, but only after GS 37-39 for Rendezvous. No correlation between yield and antigen units occurred at any growth stage for Rendezvous (Table 4), and it was not possible to determine an antigen threshold level at a growth stage where a fungicide would have a positive effect. Both flusilazole and prochloraz increased yields of cv. Rendezvous, but this may reflect control of Septoria and mildew rather than eyespot. Better correlations were obtained for cv. Pastiche (Table 4) where an association between yield and antigen level occurred from GS 37-39 onwards. The immunodiagnostic assay provided a more precise guide at this stage than visual symptoms to yield loss, with 350-700 antigen units per stem equating with a yield reduction of 0.75 tonnes per ha. This compared with a wider range at GS 37-39 of between 20-65% stem infection or an ESI from 5-30.

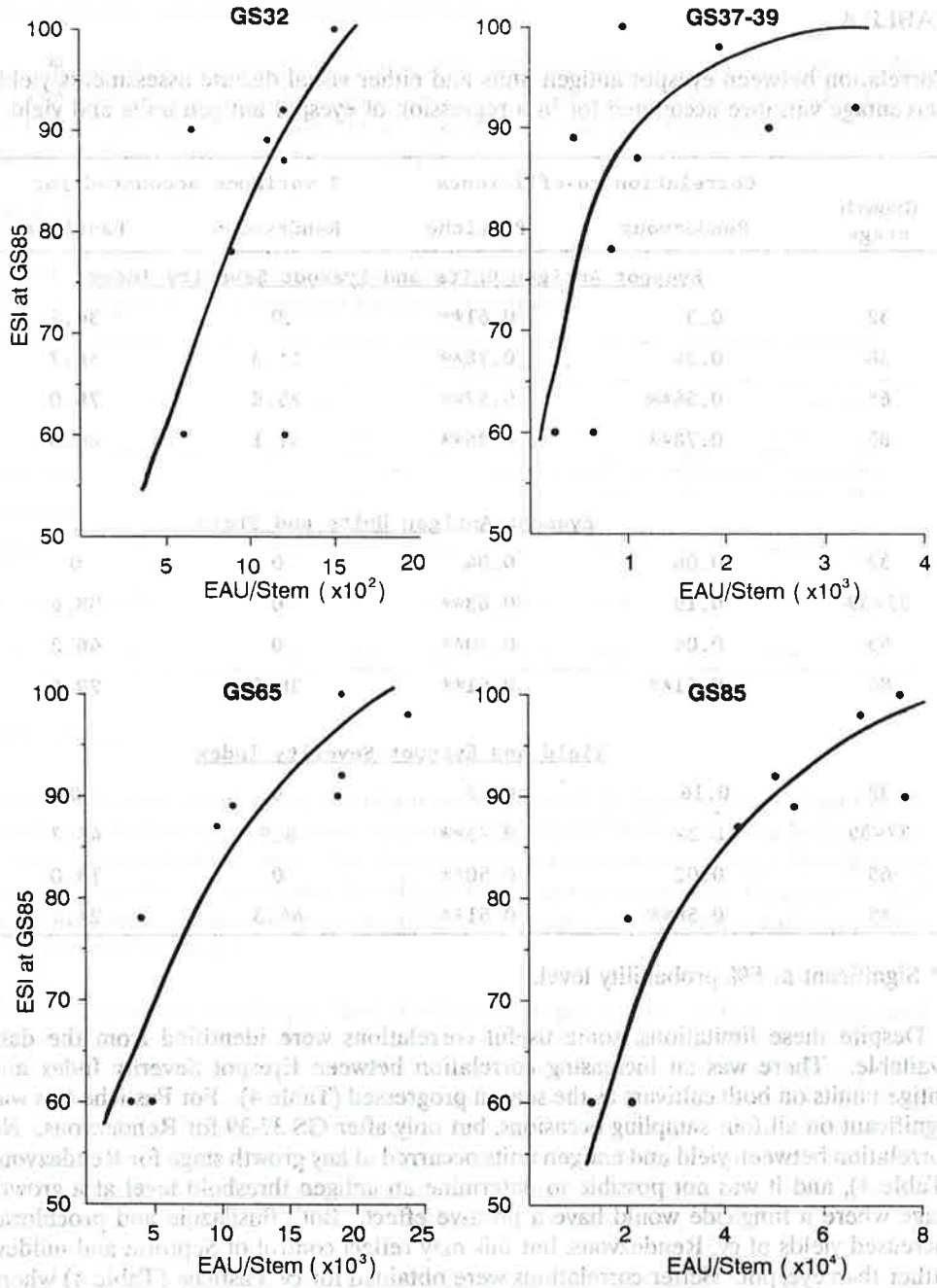

Figure Correlation between eyespot antigen units and eyespot severity index at four growth stages on the wheat cv. Pastiche. Values are for plots not treated with fungicide.

Thirty stems were routinely collected from each plot and used as a bulked sample. Immunoassays of 30 individual stems from the same plot indicated a standazrd error of 24% around the mean antigen level at a 95% confidence interval. Accuracy of thresholds could be increased at critical times by larger samples, but to double accuracy 120 stems would be needed which is, perhaps, impractical. From this one year's data it was not possible to identify a spray threshold at any growth stage, since infection levels as measured by EAUs, were high enough to justify treatment of both cultivars, even on uninoculated plots. More accurate spray thresholds would have been obtained if natural background antigen levels were lower, and fungicides applied at GS 25 or even earlier. Even so, whatever the threshold, standardised conditions for sample preparation and assay are also important.

Options based on disease assessment and meteorological data for improving spray timing have been critically reviewed by Fitt *et al.* (1988). In fact, the relationship between visual assessment of eyespot at GS 30-31 and final disease levels was poor. Serological measurements revealed a similar pattern of eyespot development to that established using traditional pathological methods, at least in a susceptible cultivar. Significant correlations were obtained during the period when fungicides may be used effectively (GS32 to 37-39), between EAUs and final disease incidence (Figure) or even yield, and these correlations were always better than those based on early visual assessments. Immunodiagnostics clearly offer the potential for better, more effective, eyespot control, and experiments of the type reported here should help to define that threshold more clearly for UK conditions.

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