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THE USE OF PCR DIAGNOSTICS TO MONITOR DEVELOPMENT OF EYESPOT IN WINTER WHEAT

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THE USE OF PCR DIAGNOSTICS TO MONITOR DEVELOPMENT OF EYESPOT IN WINTER WHEAT

by

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This is the final report of a one year research project which started in September 1995. The work was funded by a grant of £12,600 from the Home-Grown Cereals Authority (Project No. 0008/1/96). The work followed a four year project (The importance and control of stem-base diseases - Project No. 0015/1/91). The report for the present project incorporates findings relevant to eyespot from the previous project.

CONTENTS

1. SUMMARY

This project aimed to evaluate the potential for using PCR diagnostics to monitor the development of eyespot in winter wheat. The duration of the project was one year but similar techniques were applied in the final season of a preceding five year HGCA project (No. 0015/1/91). This report summarises the results from both projects where relevant to this report.

Field trials were carried out at a site in East Lothian to re-examine the timing of fungicide applications in winter wheat crops to control common eyespot (*Pseudocercosporella herpotrichoides*). There was a strong association between eyespot levels and yield and weaker correlations between degree of lodging and yield and eyespot levels and lodging. The fungicides cyprodinil and prochloraz showed the most significant levels of control of eyespot when assessed visually, and these fungicides were then used the evaluate the use of PCR diagnostics to monitor the development of the rye (R) and the wheat (W) strains of the eyespot fungus as it developed in two seasons. The results of the PCR quantification were variable, but several trends emerged over the two seasons.

Eyespot control and yield benefits can be achieved with cyprodinil and prochloraz sprays. Prochloraz had to be used early in the season, during tillering, for maximum effect on eyespot levels and cyprodinil after the start of stem extension. Spraying outside the optimum window allowed the eyespot populations to recover following treatment even if initial reductions were achieved. Prochloraz applied too late could not reduce the eyespot population sufficiently to influence the levels at the end of the season. In contrast cyprodinil applied too early achieved an initial reduction that could not be maintained until the end of the season. The findings of the work suggest the potential for using sequences of fungicides to achieve season long control of the eyespot pathogen.

Both fungicides were more effective at controlling the W strain. Cyprodinil gave a more persistent reduction in R strain eyespot than prochloraz. Control of the R strain with prochloraz was initially good but the population often recovered. The optimum timing for W strain control with prochloraz was from mid-tillering to GS 31. The W strain population could recover within two months of application after control with cyprodinil so that cyprodinil was better applied as late as possible.

PCR analysis showed that the R strain predominated in both seasons but both strains declined naturally at stem extension as old leaves died and were shed from the stem base, along with their associated eyespot lesions. These was no indication of a competitive effect between the two strains, but W levels were very low in comparison to the R strain. PCR and visual assessments up to stem extension were not useful in determining eyespot levels at the end of the season so thresholds for treatment were not successful. In one season there was a significant correlation between W strain levels at stem extension and the final levels at the end of the season, indicating how thresholds may have been more effective when the W strain was the dominant strain of eyespot in the UK.

This project highlights the use of PCR diagnostics as a tool for understanding whether treatments are effective, and why they were successful. Using the technique it was possible to

chart the initial efficacy of the fungicides following application, and the duration of control. The work demonstrated that where reductions in eyespot levels, measured as the amount of fungal DNA present, were large enough and persistent enough, eyespot levels at the end of the season were reduced. The technique demonstrated how the eyespot population was able to recover following treatment so that the most successful treatments at reducing eyespot DNA and the severity of visual symptoms at the end of the season were those that had not only reduced eyespot DNA levels initially, but had also been able to maintain this reduction for two or three months. Although in every case levels of eyespot DNA increased following the initial reduction after fungicide application, levels of eyespot DNA at the end of the season were sometimes still lower than in the untreated controls. Where this was not the case and DNA levels were not lower at the end of the season, a reduction in the level of visual symptoms and yield benefits could be demonstrated for those fungicide treatments where the initial reduction in DNA levels following treatment had been large enough and persistent enough. Because of this the work would also indicate that after symptom development PCR assessments should always be interpreted with the visual symptom data.

2. INTRODUCTION

A complex of diseases infects the stem base in wheat of which eyespot, caused by the organism *Pseudocercosporella herpotrichoides,* is the most common and the most damaging. The severity of disease development as a result of infection by common eyespot *(Pseudocercosporella herpotrichoides)* is determined by agronomic as well as environmental factors, and is greatest under cool, moist conditions and where wheat and / or barley is grown in close rotation. Eyespot is conventionally controlled in winter wheat crops with a fungicide spray at early stem extension between growth stages (Zadoks) 30 to 32 (Anon, 1987), often applied as a split treatment.

The eyespot fungus survives in the soil in crop debris, where it can persist for more than a season so that a two year break from cereals is required for effective rotational control. Eyespot is worse where cereals are grown continuously or in short rotations. Conidia are spread to the host plant by rain splash where the mycelium penetrates the coleoptiles or leaf sheaths of the host plant. Infection is localised at the stem base; it seldom infects above the second node and does not colonise leaf or root tissue. The infection can proceed through several leaf layers to eventually penetrate the stem. After infecting the stem the characteristic eye or pupil shaped lesion can form. Surrounding tissue becomes discoloured. The development of the disease is favoured in the UK by mild, wet weather in winter and cool damp weather in spring. Eyespot is most severe in early-sown crops and can be reduced in high risk fields by late sowing and crop rotation (Cook *et al.*, 1993).

There are several pathotypes of the eyespot fungus of which two commonly occur in the UK, one the W strain is highly pathogenic on wheat, but less so on barley and on rye, the second the R strain is equally pathogenic on wheat, barley and rye (Scott *et al.*, 1975). Early work on eyespot control was carried out on the W strain which predominated at the time. The fungicides most commonly used on wheat are members of the DMI group which act differentially on the two strains, and are far more effective in controlling the W strain. The R strain now predominates in many areas (King & Griffin, 1985), and this could be one reason why strategies for controlling eyespot are so ineffective. There are differences in how the two strains infect host plants, with the R strain having a more random and slower initial phase of growth than the W strain which grows faster after germination (Daniels, 1993 (a)). The R strain will invade all cell parts after they have penetrated the host whereas the W strain will only infect the cell wall. After the formation of infection plaques R strain plaques are more compact and symmetrical in comparison to the W strain, probably due to its slower growth. R strain isolates develop more slowly on leaf sheaths and on stems than W strain isolates (Goulds & Fitt, 1990). They are therefore more likely to cause lesions that only become severe later in the season.

The yield loss associated with eyespot infection and its impact on crop lodging is unclear. Sutherland & Oxley (1993) found that early fungicide use at GS 31 did not always result in an increase in yield. Clarkson (1981) found a correlation between eyespot severity and individual plant yield loss. Scott & Hollins (1978) also showed a relationship between eyespot and yield, but reported that the correlation between yield loss and lodging was stronger.

Trials carried out by SAC in the course of an HGCA funded project looking at the biology and control of eyespot (Project No. 0015/1/91) found that there was a significant association between eyespot levels and yield. Although lodging was also shown to be associated with yield loss, the correlation was not as strong as that between eyespot and yield. There was also a significant correlation between eyespot and lodging (Burnett & Oxley, 1996).

Trials to examine the fungicide timing to achieve optimum control found that by the conventional timing of stem extension the optimum timing for eyespot control had already passed for both split treatments and single sprays (Burnett & Oxley, 1996). In the same studies prochloraz gave superior control of both eyespot and lodging than flusilazole, but the pattern of control was similar in that mid-tillering was superior in terms of eyespot and lodging control for the split treatments evaluated. The 'window' for lodging control appeared to be narrower and control declined more rapidly as stem extension approached for the flusilazole sprays. Griffin (1994) reported that flusilazole provided equivalent or superior control to prochloraz on many occasions. Flusilazole is most active against the W pathotype in the eyespot population, however testing of the pathotypes at the sites used in this work showed the populations were predominantly R strain.

Single full dose sprays of prochloraz applied at GS 13 to GS 22 as early as December gave significant control of eyespot as well as a yield benefit. While this may not represent a cost-effective option in terms of application costs there is perhaps potential for a seed treatment with activity at this time. Griffin (1994) also reported superior control from early sprays as compared to later sprays at English sites where significant eyespot symptoms developed and foliar diseases were not a complicating factor. The success of early treatments could be due to successful targeting of infection sites prior to stem extension. For the multiple prochloraz sprays there was a strong negative correlation between eyespot and yield

As treatment decisions have to be made early in the season, disease risk assessment and prediction has been the aim of many research projects, with the aim of determining a threshold level of eyespot early enough in the season to identify crops where control of eyespot would be economic. Some schemes have relied on weather data, but this does not allow for the loss of lesions that either die out or are shed with the outer leaves and never penetrate the stem. The ADAS scheme for identifying crops at risk of eyespot is based on assessing the number of stems infected at the start of stem extension and recommending treatment if an incidence of more than 20% is found (Anon, 1987). This threshold was developed when the W strain of eyespot predominated whereas the R strain is now as common (King & Griffin, 1985).

Until now eyespot infection could only be accurately quantified visually. Visual differentiation between other diseases of the stem base such as sharp eyespot and Fusarium was often difficult. In addition it was only possible to differentiate between the two eyespot strains using conventional mycological techniques which were often not definitive and could not quantify the levels of each pathotype present. Techniques developed at the John Innes Centre enable the type and quantity of each pathotype to be determined by extracting the pathogen DNA from the host tissue (Nicholson & Rezanoor, 1994) and it became possible to study the differential effect that different fungicides had on the eyespot pathotypes. It was also possible to plot the levels of each pathotype throughout the season and to study how they

fluctuated following fungicide application. Control with fungicides in trials had often given rise to conflicting results, and thresholds early in the season often correlated poorly with levels of eyespot found at harvest.

Objectives

The aim of this work therefore was to utilise the novel technology available, to study the progress and development of the two strains of the eyespot fungus through a season using PCR diagnostics, and to evaluate how the progress of the two pathotypes was affected following applications of the fungicides prochloraz and cyprodinil. These two fungicides had been found to be the most effective available in controlling visual eyespot in the preceding HGCA funded study. The ability to use new technology to quantify the amounts of each pathotype present was utilised to study how the populations altered when the fungicides were used at different timings through the season, in the hope that a fungicide and timing combination for optimum control could be identified. The duration of the project was one year but similar techniques were applied in the final season of the preceding five year HGCA project (No. 0015/1/91). This report summarises the results from both projects where relevant to the PCR evaluation.

3. MATERIALS AND METHODS

The field trials were conducted on winter wheat over five seasons from 1991/1992 to 1995/1996. The first four years were the subject of a separate HGCA project (No. 0015/1/91) and evaluated a series of four, three, two and single spray programmes of prochloraz or flusilazole at approximately fortnightly intervals during tillering and stem extension, evaluated in 1991/1992 and 1992/1993. In 1993/1994 the programme of sprays was extended to include the fungicide cyprodinil. The programme in 1994/1995 and 1995/1996 was simplified and single prochloraz or cyprodinil sprays were applied at monthly intervals (Table 1). Full details of the first three years' trials are appended (Appendix One) in the form of a paper presented at the Crop Protection in Northern Britain Conference in 1996. The data on cyprodinil as applied in 1993/1994 were not presented in this paper and are detailed below.

The variety Beaver was sown in the first three years of trials and Riband was used for the 1994/1995 and 1995/1996 seasons. The trials were located in East Lothian and were all sited in commercial second wheats, apart from the 1994/1995 trial which was in a first wheat after a one year break of winter oilseed rape, and was therefore still classed as at risk from eyespot. Plots were approximately 40 m^2 and were laid out in randomised blocks. There were three replicates of the treatments in the first three seasons, and this was increased to four in the last two seasons, because of the variable distribution of the eyespot. Fungicide treatments were applied using a hand-held Cooper Pegler CP3 sprayer calibrated to deliver a water volume of 200 l/ha at a pressure of 2.5 bars. All plots including the untreated were over-sprayed with fungicides at GS 39 and GS 55 (Tottman & Broad, 1987) to eliminate foliar disease development. Plots were not oversprayed in the final season of the trials. Except for fungicides the trial areas received the same inputs as the surrounding commercial crop.

Visual stem base disease assessments were carried out by sampling twenty five tillers per plot and recording a percentage disease severity index as follows:-

$$\frac{((\text{no. slightly infected stems})+(\text{no. moderately infected stems} \times 2)+(\text{no. severely infected stems} \times 3))\times 4}{3}$$

The stem base diseases common eyespot, sharp eyespot and stem base browning Fusarium spp. were assessed at each sampling. Lodging (percentage of each plot leaning at more than 45°) and yield (tonnes per hectare corrected to 85% moisture content) were assessed at harvest. The sampling dates and crop growth stages are detailed in Table 2, 4 and 6 and the spray programmes evaluated, in Table 1, 3 and 5.

Detection of *Pseudocercosporella herpotrichoides* in wheat stem base tissue by PCR

Methodology

In the 1994/1995 and the 1995/1996 season PCR diagnostics were used to study the progress of the eyespot epidemic, in conjunction with the visual assessments. At each sampling date, 25 stem bases were chosen at random from each of four replicate plots. Early in the season, prior to stem extension, one stem base was defined as being one plant, but later samples took

the form of 25 tillers from different plants. Roots (also the crown root and seed coat if still attached) were removed close to the crown and the stem base was cut to 2 - 3 cm in length. The upper part of the plant and any remaining leaf laminae were discarded. Tissue was rinsed in tap water followed by distilled water, transferred to plastic weighing boats, covered in clingfilm then frozen at -80°C until freeze-drying could be carried out. Samples were removed from the -80°C freezer, still frozen, then placed on the freeze-dryer for 48h (the clingfilm was pierced first). The tissue was removed to plastic storage boxes containing silica gel and stored at -80°C until DNA could be extracted.

Prior to DNA extraction, the freeze-dried weight of each pooled 25 stem base-sample was recorded. The sample was transferred to a pestle and mortar and ground in liquid nitrogen to a fine flowable powder. This was removed to a centrifuge tube and DNA extracted using a commercially available kit designed for plant DNA extraction (Nucleon Phytopure, Scotlab Ltd, Coatbridge, Strathclyde). Final re-suspension of the DNA was made in 500µl TE (tris-EDTA buffer pH 8.0) in plastic eppendorf tubes. Primers were applied to aliquots of the samples for detection of W- and R-strain *P. herpotrichoides*. A competitive PCR technique was used which enables quantification of PCR products; details of the competitive PCR process used have been submitted for a patent application and are therefore confidential. Results were expressed in 1996 as µg fungal DNA per unit dry weight of stem base and used to quantify the amount of each fungus pathotype present at each sampling date. Results can be compared within years, but the probes were modified between 1995 and 1996 so that between season comparisons are not valid.

TREATMENTS AND ASSESSMENTS

Full commercial doses for the products used were as follows:-

Active ingredient	*Product*	*Manufacturer*	*g a.i./ha*
P = prochloraz	Sportak 45	AgrEvo	405
F = flusilazole	Sanction	DuPont	200
C = cyprodinil	Developmental	Novartis	1000

Treatments applied by CO^2 knapsack sprayer in 200 - 250 litres of water/ha at 200 -300 kPa Zadoks growth stages (Tottman & Broad, 1987).

1993 / 1994 season

Table 1.
Eyespot spray programmes evaluated on winter wheat trials 1993/1994
Treatment regimes 1 - 10

Spray date	Growth stage	1	2	3	4	5	6	7	8	9	10
22/2/94	13-21	-	P	-	-	F	-		C	-	-
22/3/94	14-23	-	-	P	-	-	F		-	C	-
19/4/94	24-27	-	-	-	P	-	-	F	-	-	C

Table 2.
Assessments dates and growth stages 1994

Assessment	Sampling date	Growth stage
Assessment 1	8/2/94	12
Assessment 2	25/2/94	13-21
Assessment 3	9/3/94	13-21
Assessment 4	21/3/94	14-23
Assessment 5	5/4/94	14-23
Assessment 6	19/4/94	24-27
Assessment 7	4/5/94	24-27
Assessment 8	16/5/94	32
Assessment 9	13/6/94	49-51
Assessment 10	18/7/94	71
Assessment 11	10/8/94	85-87

1994 /1995 season

Table 3.

Eyespot spray programmes evaluated on winter wheat trials 1994/1995
Treatment regimes 1 - 11

Spray date	Growth stage	1	2	3	4	5	6	7	8	9	10	11
5/12/94	13-21	-	P	-	-	-	-	C	-	-	-	-
14/2/95	21-22	-	-	P	-	-	-	-	C	-	-	-
14/3/95	25-27	-	-	-	P	-	-	-	-	C	-	-
14/4/95	30	-	-	-	-	P	-	-	-	-	C	-
1/5/95	32	-	-	-	-	-	P	-	-	-	-	C

Table 4.
Assessment dates and growth stages 1995

Assessment	Sampling date	Growth stage
Assessment 1	7/12/94	13-21
Assessment 2	7/2/95	21-22
Assessment 3	13/3/95	25-27
Assessment 4	11/4/95	30
Assessment 5	8/5/95	32
Assessment 6	22/6/95	59-61
Assessment 7	8/8/95	87

1995 / 1996 season

Table 5.
Eyespot spray programmes evaluated on winter wheat trials 1995/1996
Treatment regimes 1 - 11

Spray date	Growth stage	1	2	3	4	5	6	7	8	9	10	11
20/12/95	12-22	-	P	-	-	-	-	-	-	-	-	-
21/2/96	24	-	-	P	-	-	-	PC	C	-	-	-
18/3/96	24-25	-	-	-	P	-	-	-	-	C	-	-
26/4/96	31	-	-	-	-	P	-	-	-	-	C	-
15/5/96	33	-	-	-	-	-	P	-	-	-	-	C

Table 6.
Assessment dates and growth stages 1996

Assessment	Sampling date	Growth stage
Assessment 1	21/12/95	12-22
Assessment 2	22/02/96	24
Assessment 3	18/03/96	24-25
Assessment 4	22/04/96	31
Assessment 5	14/05/96	33
Assessment 6	15/08/96	85

4. RESULTS

The data from the trials are presented below and in Appendix Two. Only eyespot levels at stem extension and late in the season are presented in this section for clarity, and the remaining eyespot assessments and results of visual assessments for *Fusarium* spp. and sharp eyespot are shown in Appendix Two.

1994

Table 7.
Visual eyespot indexes and yield in 1994

		Growth stage of assessment				
Treatment	Treat /GS*	32	49/51	71	85/87	Yield
T1	UT	6.7	1.3	26.7	80.0	10.5
T2	P-21	4.0	5.3	14.7	46.7	10.3
T3	P-23	4.0	5.3	22.7	52.0	10.2
T4	P-27	5.3	8.0	12.0	52.0	10.7
T5	F-21	2.7	6.8	24.0	78.7	10.5
T6	F-23	8.0	4.0	33.3	65.3	10.3
T7	F-27	4.0	4.0	10.7	50.7	10.9
T8	C-21	2.7	5.3	10.7	52.0	10.5
T9	C-23	9.3	6.8	32.0	45.3	10.7
T10	C-27	2.7	6.8	21.3	57.3	10.5
SED		0.26	5.72	12.81	27.69	0.30
d.f.		27	27	27	27	27

* P = prochloraz, C = cyprodinil, F = flusilazole - growth stage applied

1994 was the first year that cyprodinil was included in the trial series and evaluated for eyespot control. The indices for visual eyespot at growth stage 85/87, just prior to harvest, and the yields are shown in Figures 1 and 2. Eyespot levels at stem extension were a poor indicator of levels in the plots at the end of the season (Table 7) with no significant correlation between the two. Prochloraz gave optimum control of eyespot when applied early in the season in February, but was good at all three timings. Flusilazole showed best control of eyespot at the latest spray timing and was poor at the earlier timings (Figure 1). Control with flusilazole was better in this season than in previous seasons (Appendix 1). In this season early sprays in February did not show a yield benefit. Cyprodinil gave comparable control to prochloraz at each spray timing, and a better level of control over the three timings than flusilazole. Cyprodinil applied in March gave a yield benefit over the untreated plots.

1995

Table 8.
Visual eyespot indexes and yield in 1995

		Growth stage of assessment				
Treatment	Treat /GS*	32	59/61	87	Lod 5	Yield
T1	UT	2.0	18.3	49.7	37.5	9.9
T2	P-21	0.2	10.3	22.0	6.0	10.9
T3	P-22	0.8	10.3	17.7	6.5	11.0
T4	P-27	1.5	12.7	21.0	4.8	11.0
T5	P-30	1.0	7.0	25.7	4.8	11.1
T6	P-32	1.8	15.0	23.7	7.0	10.7
T7	C-21	1.8	13.7	35.7	22.5	10.5
T8	C-22	1.8	17.0	41.0	16.2	10.7
T9	C-27	0.8	17.0	44.0	13.2	10.5
T10	C-30	0.8	13.0	41.3	21.2	10.7
T11	C-32	0.8	6.3	31.0	27.0	10.9
SED		0.60	2.87	5.43	8.12	0.29
d.f.		43	43	43	43	43

* UT = untreated, P = prochloraz, C = cyprodinil - growth stage applied

Prochloraz applied early in the season at mid-tillering gave the best control of visual eyespot and the greatest yield benefit (Table 8). Eyespot control with prochloraz declined as spray applications were made later in the season. Optimum control and yield benefit with cyprodinil was achieved later in the season at the last spray timing made at GS 32 (Figures 3 and 4). There were large yield benefits in this season to all the spray applications and timings. The largest yield benefit from prochloraz was as a result of any of the mid-tillering up to the start of stem extension sprays.

1996

Table 9.
Visual eyespot indexes and yield in 1996

Treatment	Treatment / growth stage	Growth stage of assessment 31	33	85	Yield
T1	UT	1.2	1.7	26.1	11.8
T2	P-22	1.8	1.3	15.7	11.0
T3	P-24	1.0	2.0	10.7	11.1
T4	P-25	0.8	0.7	21.0	11.0
T5	P-31	1.2	1.7	19.3	10.6
T6	P-33	2.2	1.3	21.0	11.3
T7	C-22	1.0	2.7	15.3	11.5
T8	C-24	2.2	0.7	24.3	11.3
T9	C-25	1.0	0.0	26.0	10.6
T10	C-31	2.2	1.3	18.8	11.5
T11	C-33	1.8	0.7	16.3	11.7
SED		0.89	0.89	6.01	0.59
d.f.		43	43	43	43

As in the previous seasons' trials the optimum timing for eyespot control with prochloraz was earlier in the season than with cyprodinil (Figure 5). Eyespot control with prochloraz was superior at the early tillering timing, but control with cyprodinil was poor at this timing but improved steadily the later the application was applied, so that optimum control of visual eyespot was achieved with an application at GS 33. Yields were very variable in this season as foliar disease levels were high later in the season (Table 9).

PCR ANALYSIS

Table 10.
PCR analysis of eyespot 1995 (DNA µg/unit plant tissue)

Date	December		March		May		August	
Growth stage	GS 13/21		GS 25/27		GS 32		GS 87	
Treat-ment	W strain	R strain	W strain	R strain	W strain	R strain	W strain	R strain
T1	0	0	0.022	0.858	0.010	0.062	0.025	0.088
T2	0	0	0.000	0.018	0.000	0.028	0.012	0.120
T3			0.000	0.028	0.000	0.052	0.000	0.193
T4			0.000	0.143	0.000	0.048	0.005	0.208
T5					0.000	0.058	0.003	0.267
T6					0.000	0.055	0.022	0.275
T7			0.032	0.008	0.000	0.120	0.020	0.305
T8			0.022	0.068	0.000	0.040	0.012	0.370
T9			0.022	0.033	0.000	0.030	0.015	0.158
T10					0.000	0.055	0.018	0.250
T11					0.000	0.050	0.012	0.162
SED	*	*	0.0313	0.4906	0.0028	0.0378	0.0064	0.1192
d.f.	*	*	27	24	43	41	42	40
P	*	*	0.787	0.431	0.008	0.383	0.001	0.285

PCR analysis showed that the R strain predominated at each assessment timing in this season (Table 10). The two strains tended to increase and decrease in tandem, rather than for one to increase as the other decreased. There was a large peak in both strains at the March assessment in the untreated plots, and this declined at the May assessment and climbed again in August. There was no indication of any positive or negative association between the two strains however ($r = -0.069$ in March, $r = 0.026$ in May and $r = 0.175$ in August).

The amount of eyespot DNA extracted for the W strain of the eyespot fungus showed that significant control with prochloraz was achieved. Optimum control with prochloraz was achieved following a spray at early tillering, and control of the W strain remained good with prochloraz until the GS 30 timing in April (Figure 6). There were no significant differences in the degree of control of the W strain achieved with prochloraz between these spray timings. Control was not as good with a prochloraz spray applied at GS 32. This decline in effectiveness at later spray dates was mirrored in the visual assessments (Figure 3). The optimum timing for cyprodinil was achieved at the GS 32 timing when control with prochloraz was least effective.

Assessment of the R strain using PCR gave very variable results (Figure 7), and all the treatments resulted in higher levels for eyespot DNA than the untreated control at the GS 85

assessment. Early treatments of cyprodinil were the most effective, but still exceeded the levels in the untreated control plots. Early sprays of cyprodinil were particularly poor at controlling R strain eyespot levels at the end of the season. The reverse was true for prochloraz with early sprays giving the largest reductions in R and W strains two to three months after spraying as well as best control of R strains by the end of the season.

The development of the two strains are plotted in Figures 8 and 9, following the treatments applied in March at mid to late tillering. In the untreated plots the wheat strain of the fungus increased through the season with a dip at the May assessment timing. An application of prochloraz in March at mid to late tillering gave a large and significant decline in W strain levels assessed two months after the spray had been applied. Although levels had started to increase again at the end of the season they were still less than in the untreated controls. A similar significant reduction was seen following an application of cyprodinil at this timing but recovery of the population was more rapid although levels were still less than in the untreated plots at the end of the season.

The R strain on the other hand increased rapidly in the untreated plots, but then declined and remained steady later in the season. Applications of prochloraz at mid-tillering did reduce the levels of R strain compared to the untreated plots at this timing but by stem extension (GS 32) in May, two months after the sprays had been applied, levels in the treated plots had increased. By the end of the season levels in these plots had exceeded the untreated control and the cyprodinil treatments (Figure 9). The cyprodinil treatment in March at mid-tillering did not halt this increase and indeed the levels continued to rise in the cyprodinil treated plots, although not as rapidly as in the prochloraz treatment. This pattern of a reduction in R strain levels after treatment followed by an increase was seen for each treatment timing.

Figure 10 charts the development of the W strain following an application made in May at GS 32. Both prochloraz and cyprodinil show a reduction in W strain eyespot levels at the end of the season, but cyprodinil treatment at GS 32 is still providing a larger reduction in W levels at the end of the season. As with the earlier treatments the R strain population in the treated plots recovers and exceeds that in the untreated plots (Figure 11). Any reduction in eyespot levels provided by the fungicide application had disappeared by August and the recovery of the R strain population was greater after the prochloraz treatment and slower after the cyprodinil treatment.

Table 11.
PCR analysis of eyespot 1996 (DNA µg/unit plant tissue)

Date	December		February		March		April		August	
Growth stage	GS 12/22		GS 24		GS 24/25		GS 31		GS 85	
Treat-ment	W strain	R strain	W strain	R strain	W strain	R strain	W strain	R strain	W strain	R strain
T1	0.195	0.074	0.330	1.341	0.298	2.215	0.228	0.320	0.245	1.280
T2	0.381	0.086	0.271	0.289	0.115	0.136	0.192	0.235	0.536	5.758
T3			0.388	1.254	0.270	0.175	0.241	0.334	0.411	4.470
T4							0.211	0.204	0.281	6.986
T5							0.245	0.879	0.265	5.626
T6									0.322	5.286
T7			0.319	0.522	0.341	0.174	0.189	0.783	0.495	5.613
T8			0.260	1.299	0.355	0.205	0.231	0.860	0.220	2.334
T9							0.098	0.386	0.269	1.480
T10							0.205	0.878	0.676	0.534
T11									0.172	0.018
SED	0.1371	0.0345	0.1049	0.6137	0.1510	0.7412	0.0077	0.9695	0.3235	5.2915
d.f.	7	7	19	19	19	19	33	33	42	42
P	0.168	0.691	0.647	0.192	0.401	0.020	0.789	0.075	0.791	0.823

As in the previous season levels of both strains increased and decreased together with a peak early in the season and then a decline mid season and an increase by the end of the season. There was no indication of a positive or negative association between the W and the R strains ($r = -0.002$ in December, $r = 0.259$ in February, $r = 0.482$ in March, $r = 0.116$ in April and $r = 0.006$ in August). The R strain predominated in 1996 (Table 11) as it had in the 1995 season.

PCR analysis showed that the prochloraz treatments at each spray timing had not reduced the levels of wheat strain eyespot in the plots at the end of the season (Figure 12.). The lowest levels of eyespot at the end of the season as a result of prochloraz treatment was following a treatment when prochloraz was applied at the conventional timing of mid-tillering (GS 25) to early stem extension (GS 31), but levels were still higher than in the untreated control. Early sprays of prochloraz gave the highest levels of W strain eyespot. Optimum control of the W strain with cyprodinil was achieved later in the season at GS 33; a spray earlier than this in April resulted in higher levels of W strain DNA by the end of the season.

Optimum control of the R strain was achieved with a cyprodinil spray applied in May at GS 33 (Figure 13), with good control also achieved from the April application at GS 31. Control of the R strain by the August assessment was not achieved from any spray timing in this season with prochloraz - all the prochloraz treatments showed higher levels of R strain

eyespot than the untreated controls. Of all the prochloraz treatments the one applied in February at GS 24 had the lowest levels of R strain eyespot DNA at the end of the season. The assessments made in March and April, however, did show that prochloraz treatment earlier had reduced R strain levels, and differences between treatments were significant at this timing. Earlier cyprodinil sprays had not controlled the R strain at this point in the season.

The development of the two strains are plotted in Figures 14 and 15. In the untreated plots the wheat strain was present at a fairly constant level through the season. Treatment with cyprodinil in March at GS 24/25 resulted in a larger reduction in W strain levels at the end of the season than did prochloraz at the same timing, but for both fungicides the population of W recovered and eventual exceeded the levels in the untreated plots by the end of the season. The R strain, in contrast, increased steadily until March, declined naturally by the April assessment and then recovered at the end of the season. Treatment with cyprodinil caused a dip in R strain levels that persisted through to the end of the season. Treatment with prochloraz at this timing had no controlling effect and levels in this treatment exceeded those in the untreated controls.

Thresholds for eyespot treatment.

Table 12.

Correlation of eyespot at stem extension and end of season - visual and PCR 1995

	Visual eyespot GS 32	PCR GS 32 R strain	PCR GS 32 W strain	PCR GS 32 R+W
GS 87				
Visual	-0.098	-0.006	0.458**	0.206
PCR R strain		0.374*		
PCR W strain			0.143	
PCR R + W				0.357

There was no correlation between visual eyespot at GS 32 and visual eyespot at the end of the season, and no correlation between total DNA detected at GS 32 and total DNA detected at the end of the season or with visual eyespot at the end of the season. There was a significant (*P= 0.05) correlation between levels of R strain DNA detected at GS 32 and the levels of R strain DNA detected at GS 87, and a significant correlation (**P=0.01) between W strain DNA detected at GS 32 and the visual levels of disease at the end of the season. Visual assessments at the end of the season did not correlate with the levels of DNA detected (Correlation of visual eyespot and PCR R+W at GS 87 = 0.023). Analysis of the untreated plots only did not show any significant correlations (Table 13).

Table 13.

Correlation of eyespot at stem extension and end of season in untreated plots 1995

	Visual eyespot GS 32	PCR GS 32 R strain	PCR GS 32 W strain	PCR GS 32 R+W
GS 87				
Visual	-0.891	0.963	-0.367	0.704
PCR R strain		0.893		
PCR W strain			-0.957	0.329
PCR R + W				

The correlation of visual eyespot and total R+W DNA levels at GS 87 was not significant (r = 0.807).

Table 14.

Correlation of eyespot at stem extension and end of season - visual and PCR 1996

	Visual eyespot GS 31	PCR GS 31 R strain	PCR GS 31 W strain	PCR GS 31 R+W
GS 85				
Visual	-0.017	-0.227	-0.037	-0.038
PCR R strain		-0.087		
PCR W strain			0.082	
PCR R + W				0.108

Correlation of visual eyespot and PCR R+W at GS 85 = 0.023

There were no significant correlations between either visual levels of eyespot or levels detected using PCR at either GS 31 and those detected or assessed at the end of the season. Again PCR detected DNA levels did not correlate with the visual assessments made at the end of the season. There were also no significant correlations in the untreated plots taken alone.

Table 15.

Correlation of eyespot at stem extension and end of season in untreated plots 1996

	Visual eyespot GS 31	PCR GS 31 R strain	PCR GS 31 W strain	PCR GS 31 R+W
GS 85				
Visual	0.098	0.766	-0.897	0.636
PCR R strain		-0.356		
PCR W strain			-0.175	
PCR R + W				-0.156

Correlation of visual eyespot and PCR R+W at GS 85 = 0.881

Figure 1.
Visual eyespot 1994 (GS 85/87)

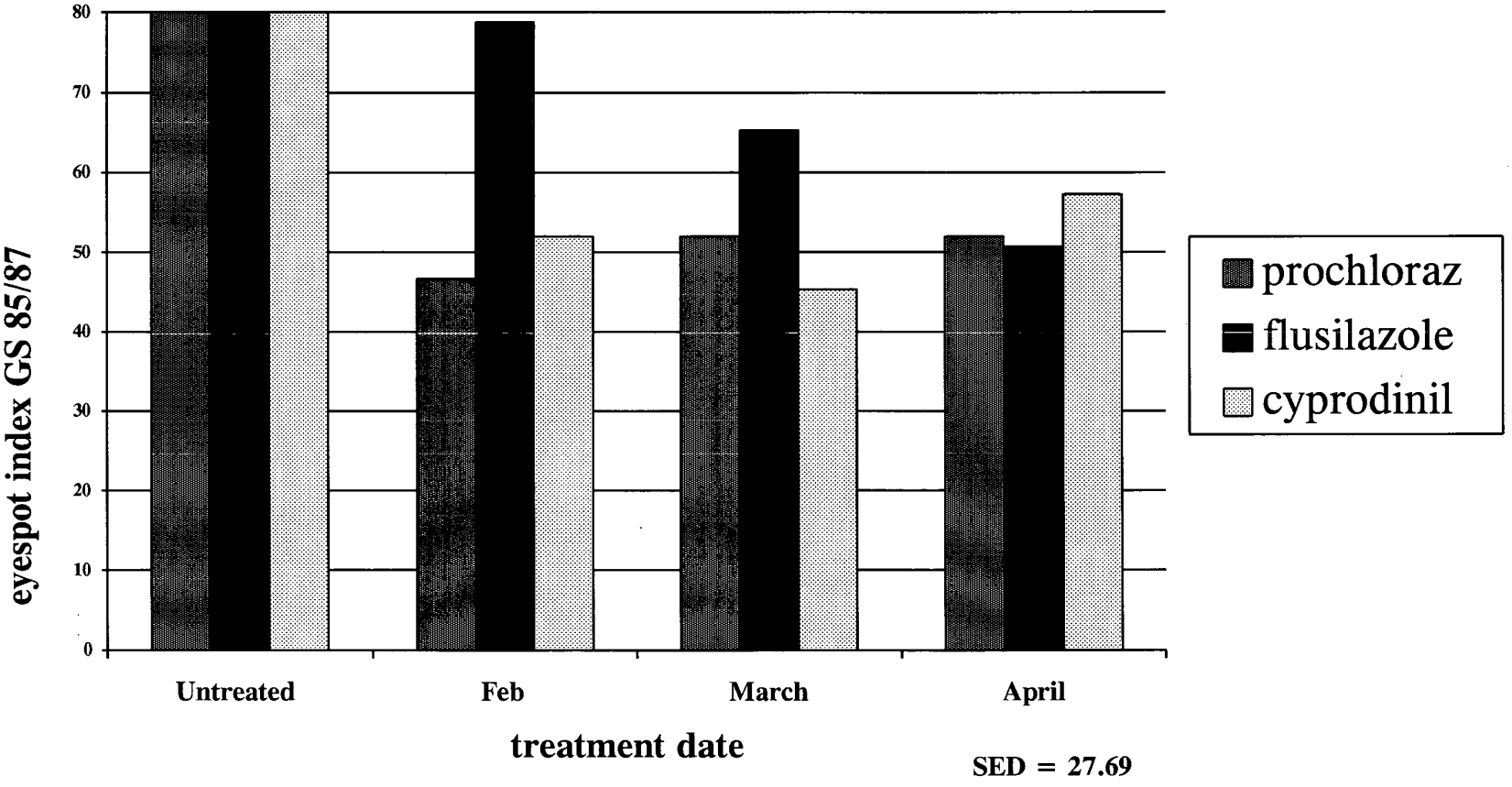


Figure 2.
Yield 1994

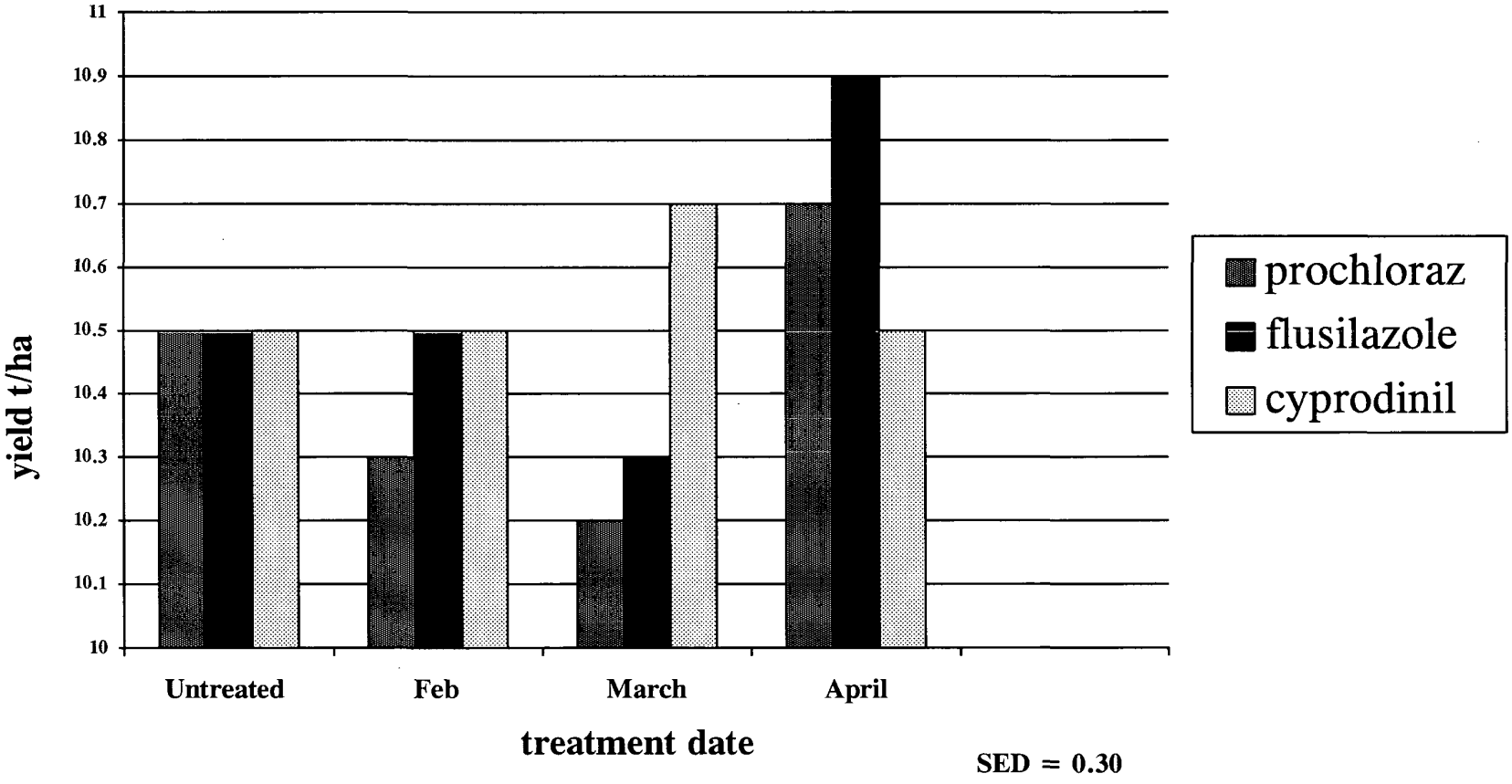


Figure 3.
Visual eyespot 1995 (GS 87)

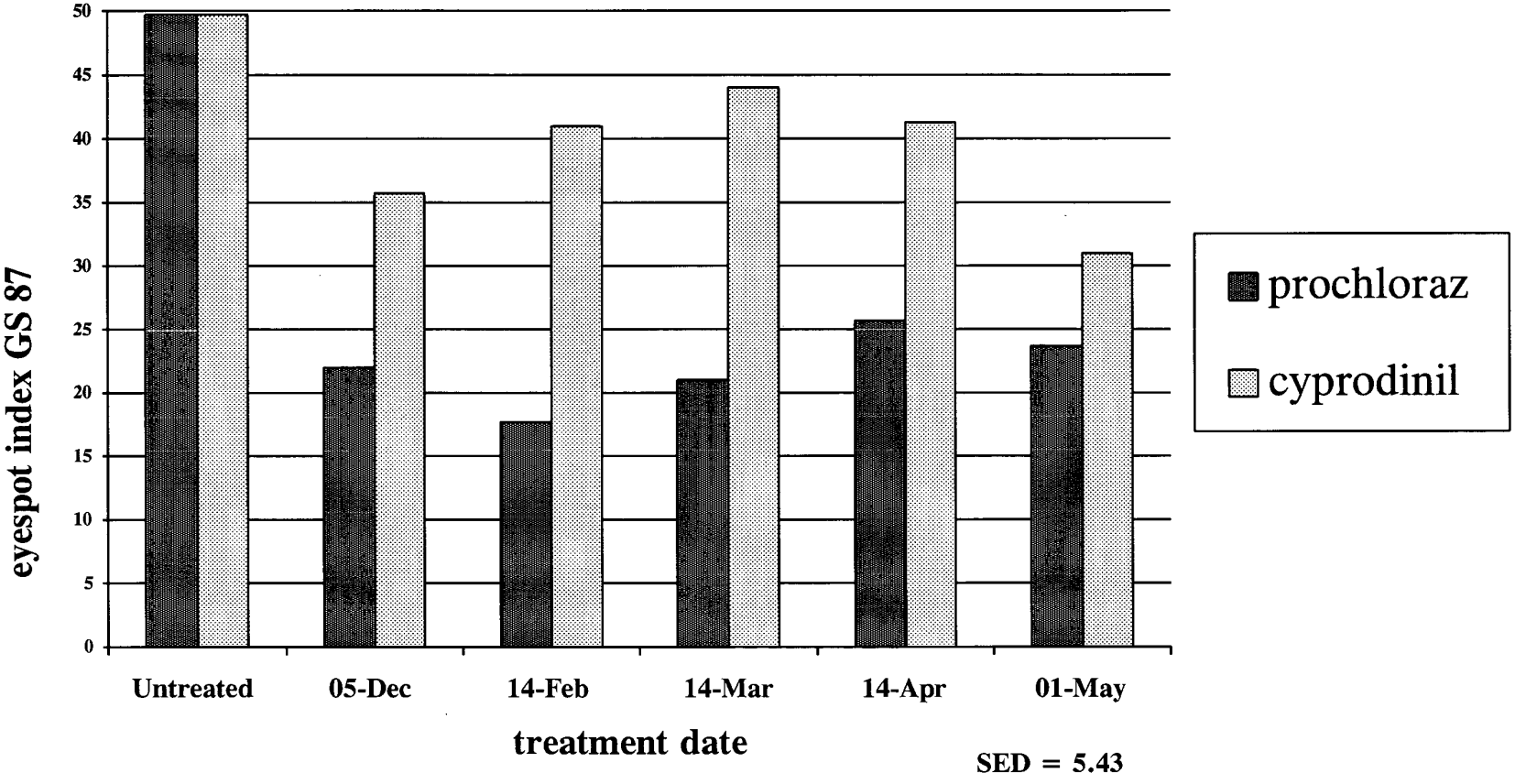


Figure 4.
Yield 1995

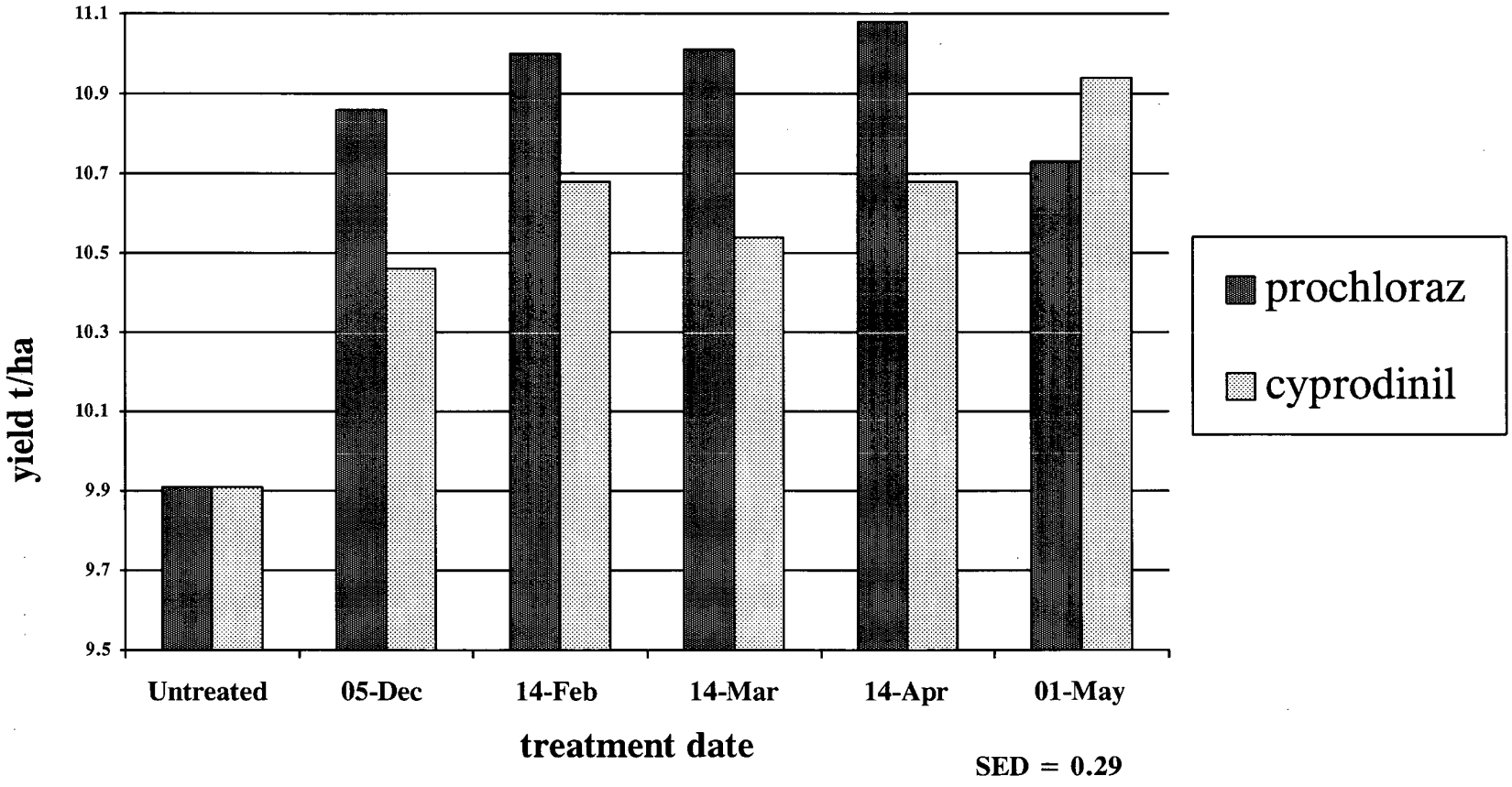
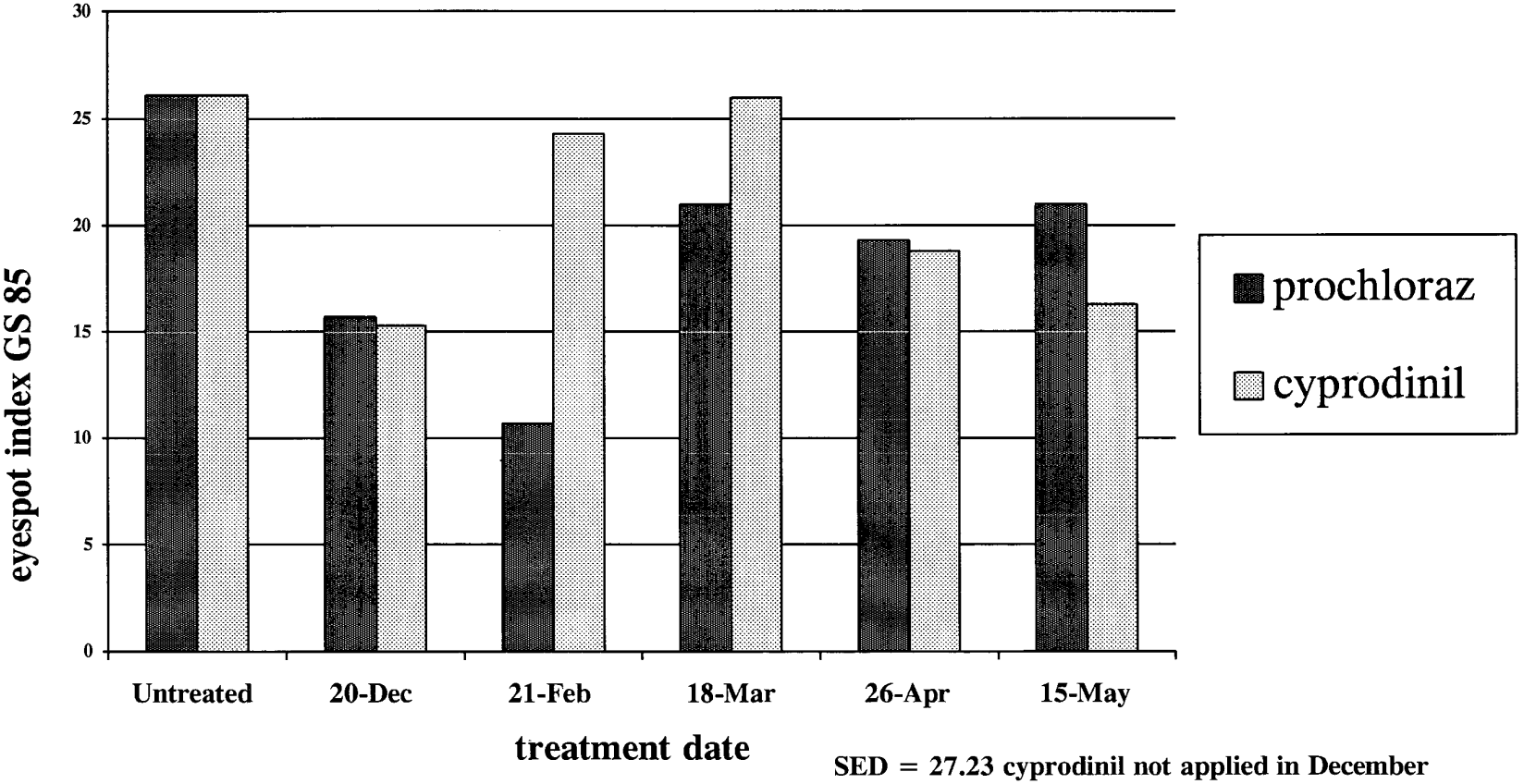


Figure 5.
Visual eyespot 1996 (GS 85)



SED = 27.23 cyprodinil not applied in December

Figure 6.
W strain eyespot 1995 at GS 87

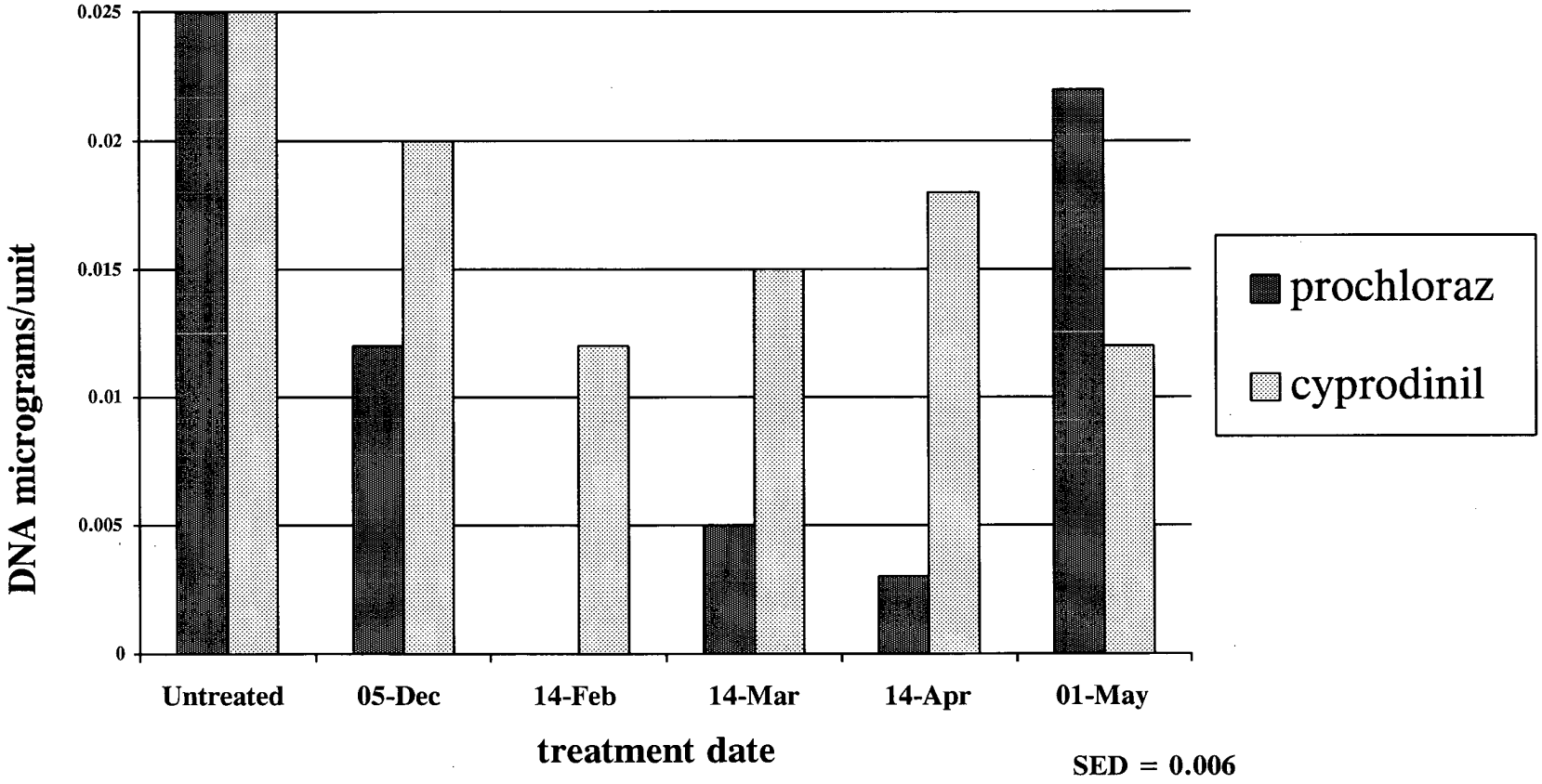


Figure 7.
R strain eyespot 1995 at GS 87

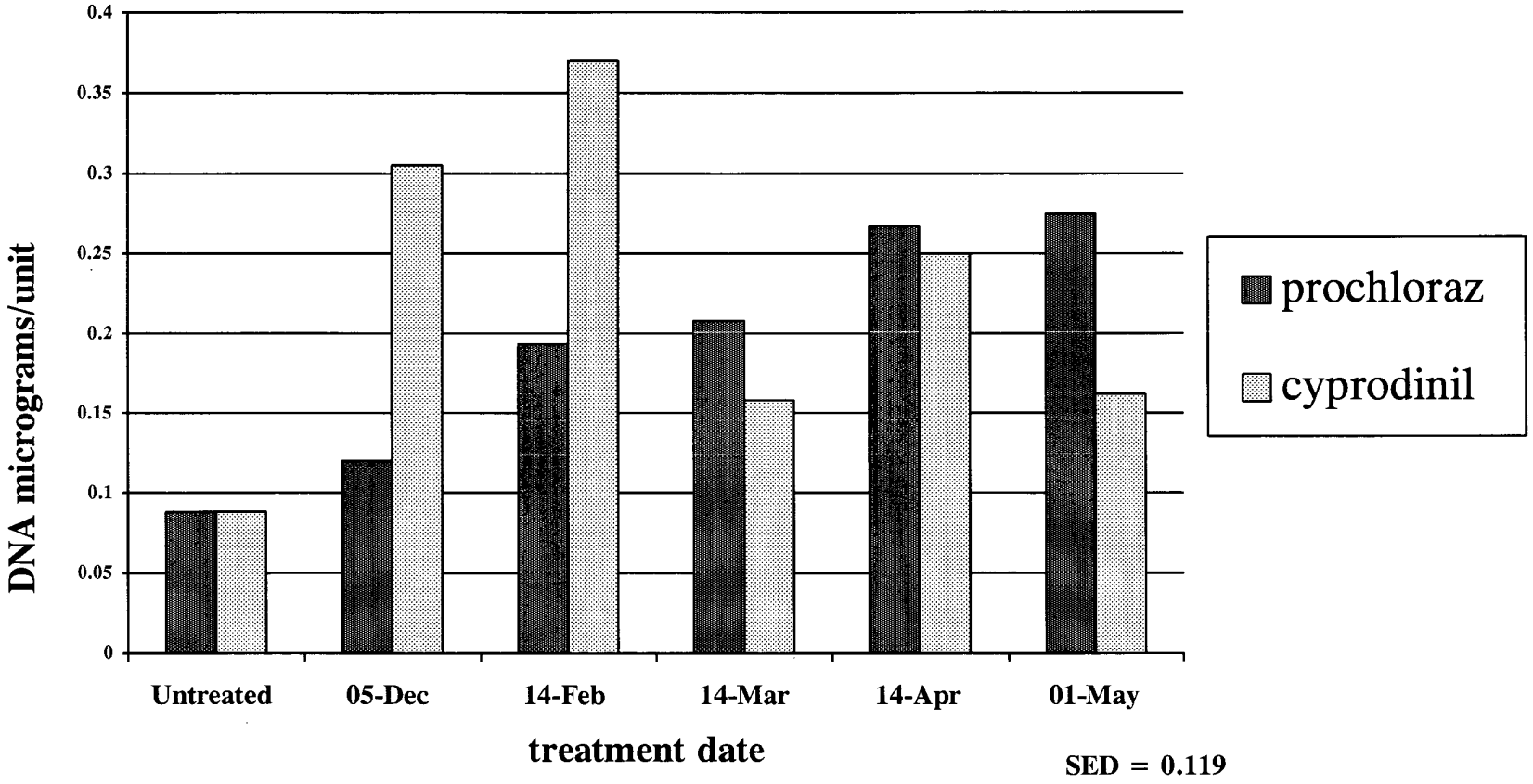


Figure 8.
Development of W strains 1995

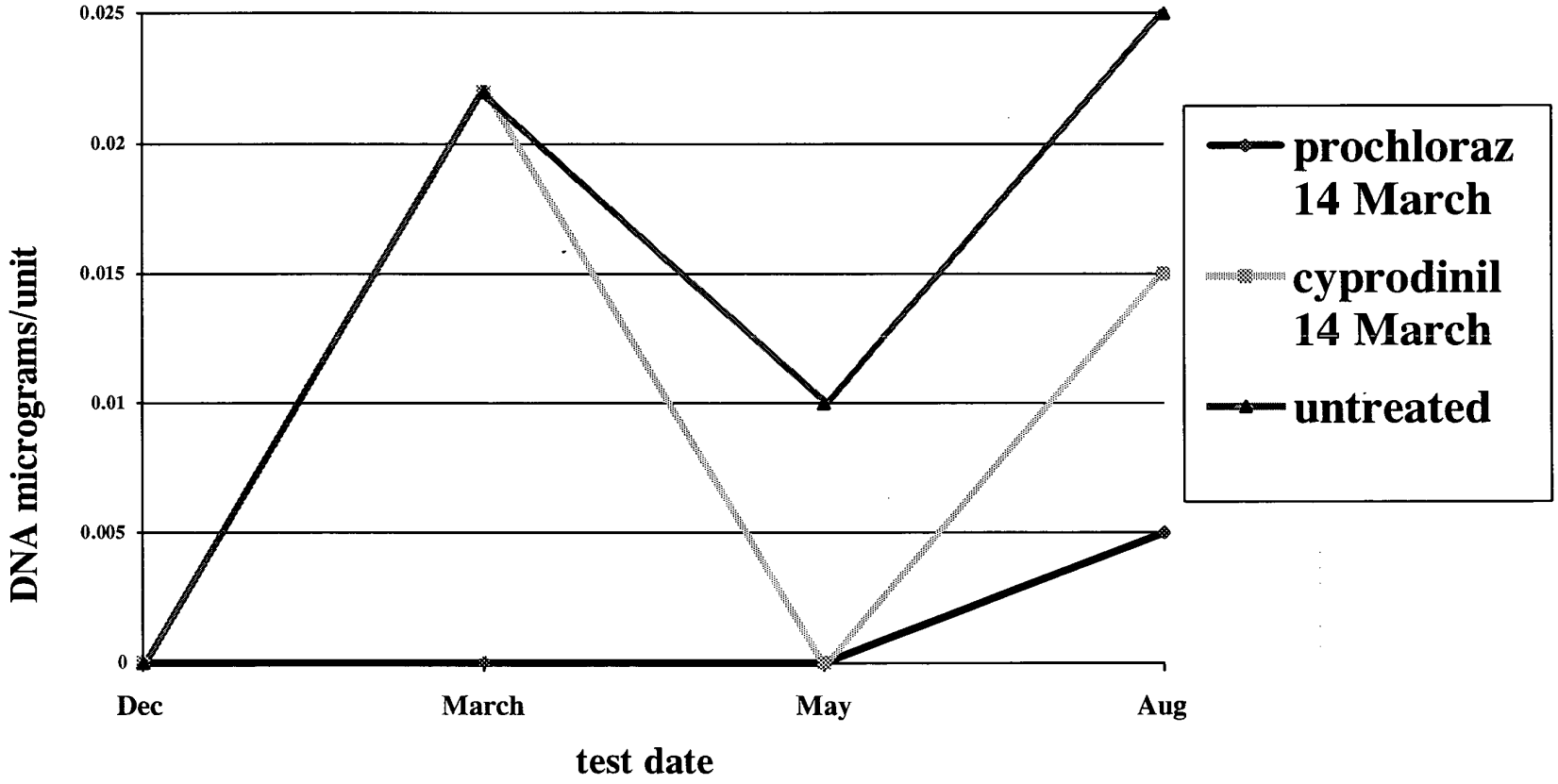


Figure 9.
Development of R strains 1995

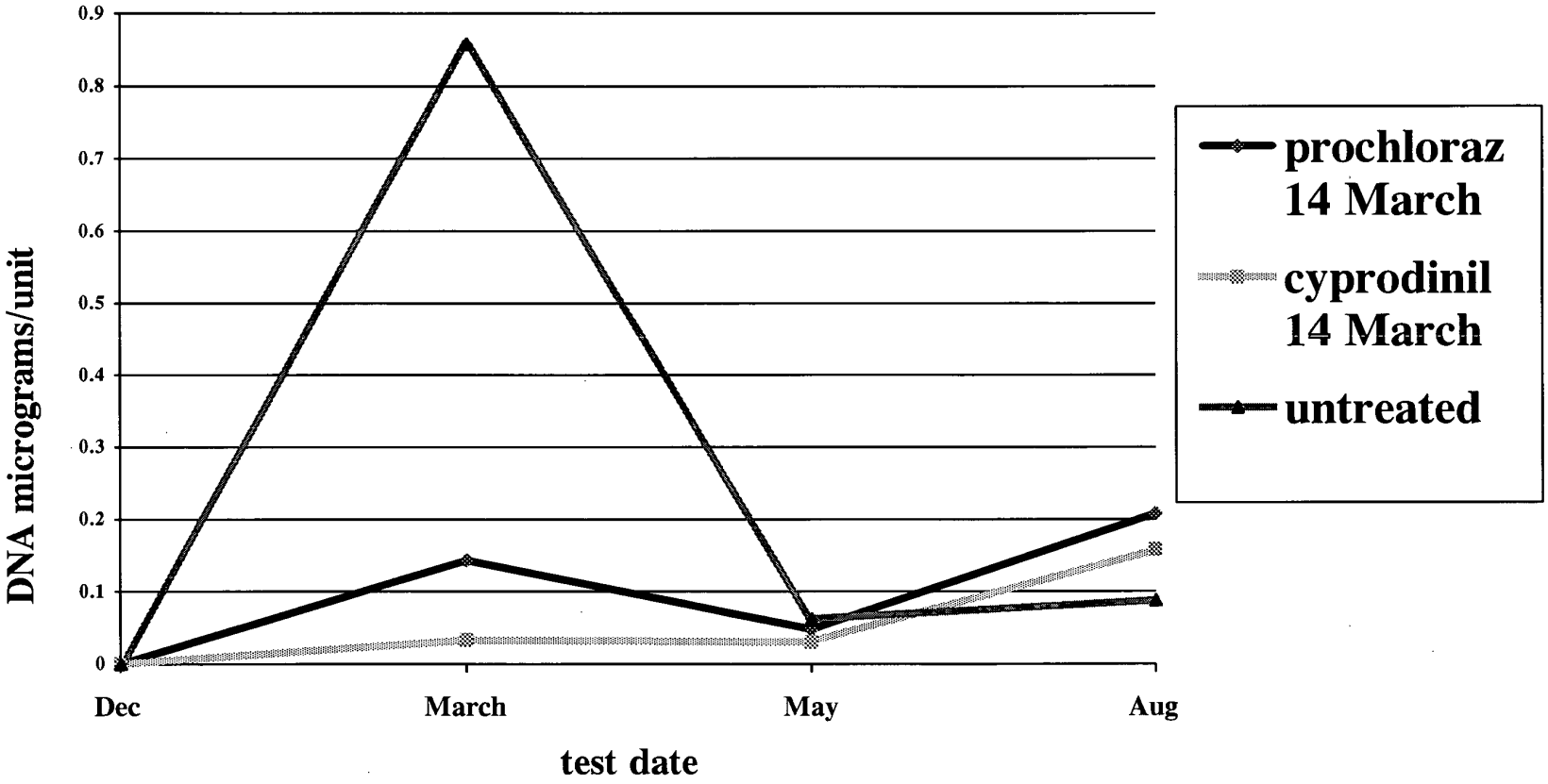


Figure 10.
Development of W strains 1995

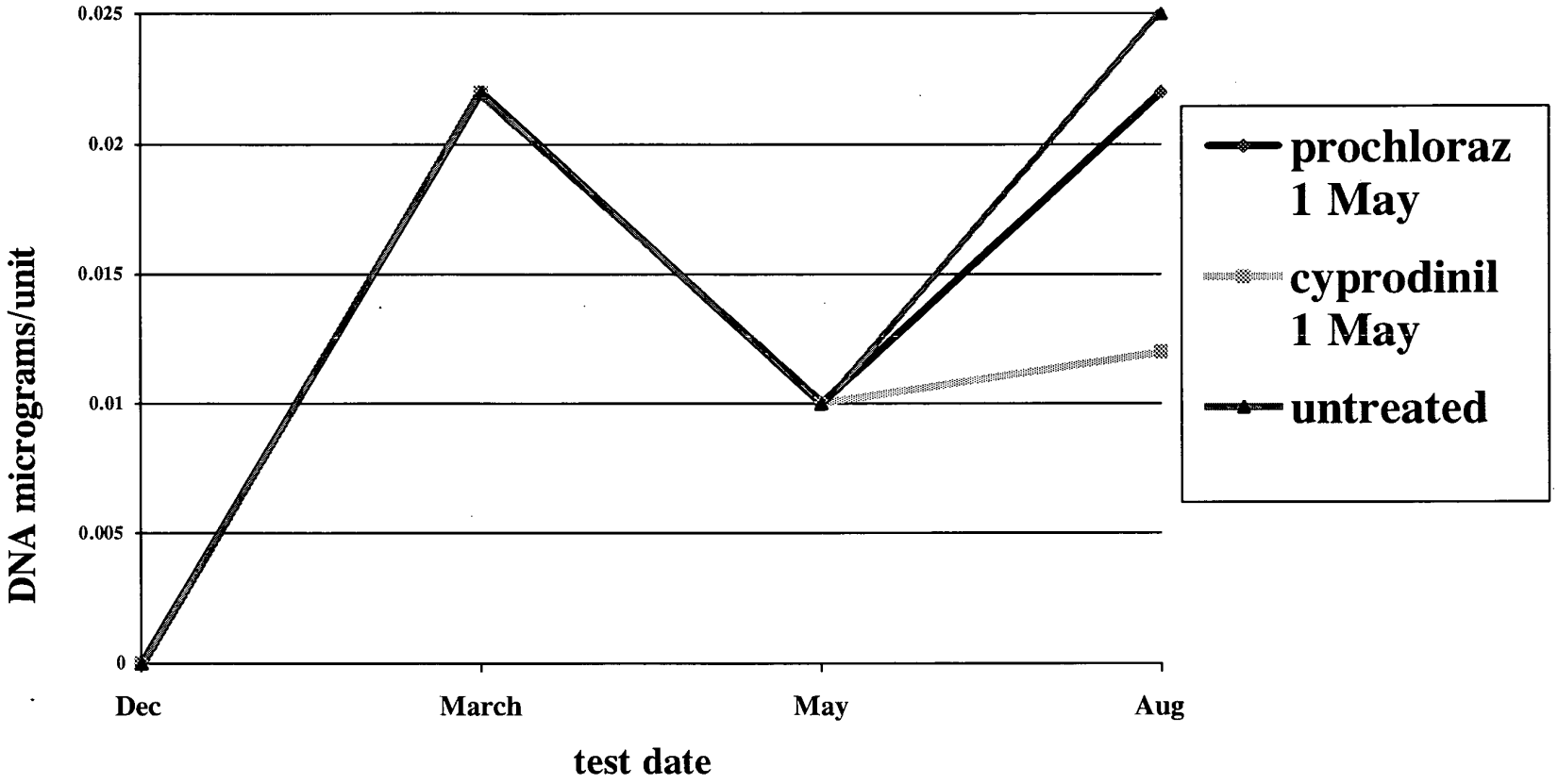


Figure 11.
Development of R strains 1995

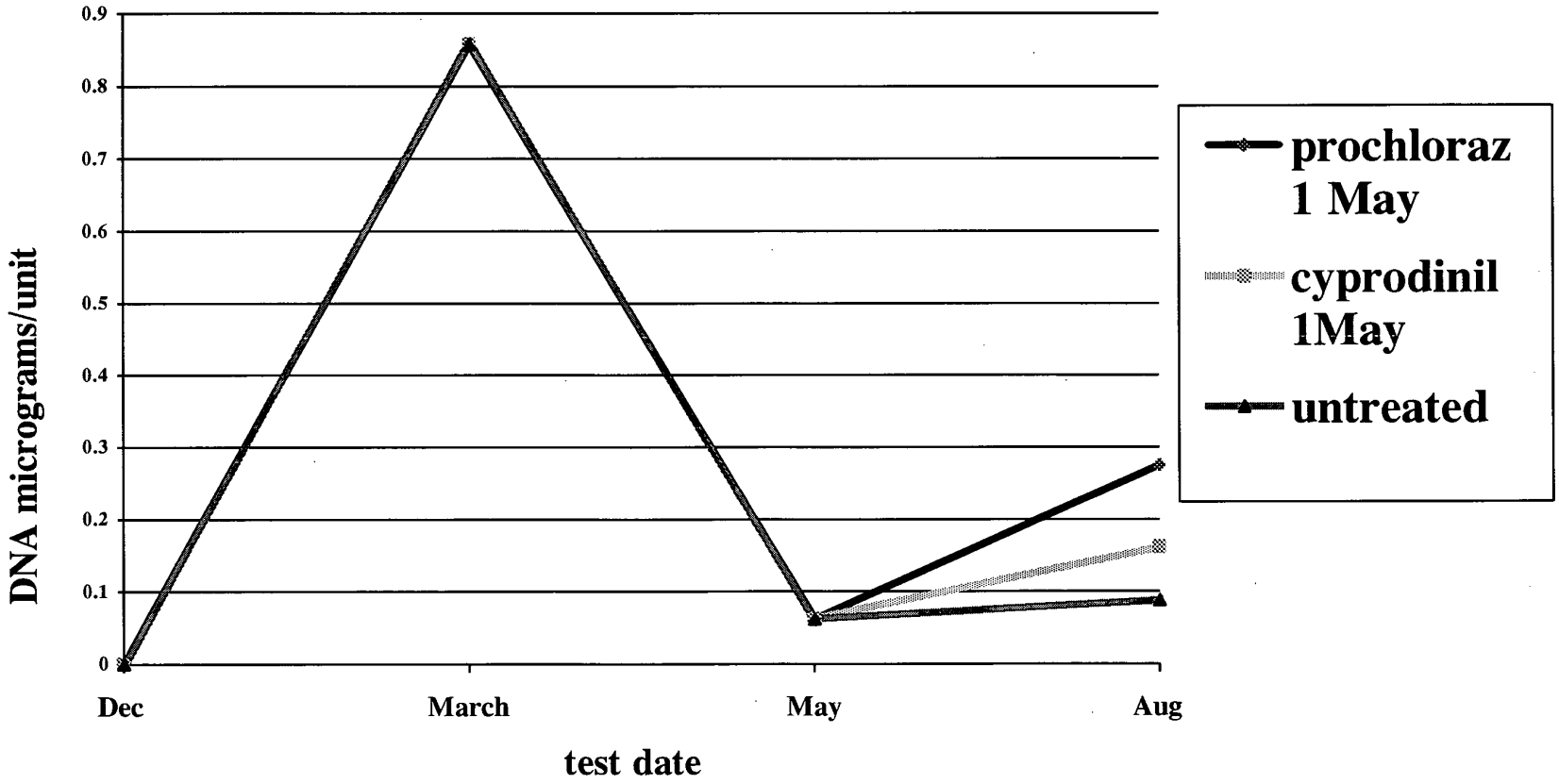
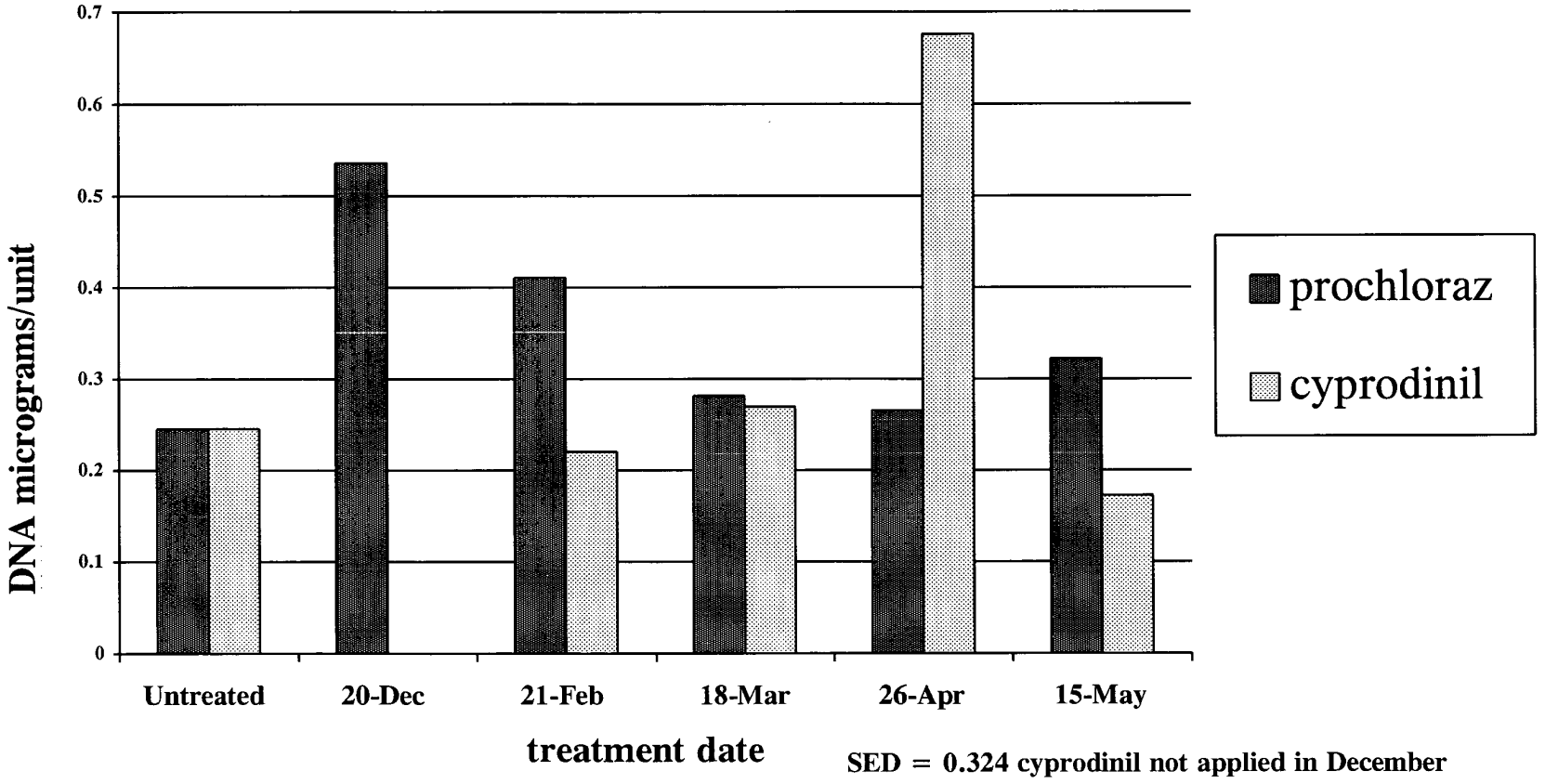
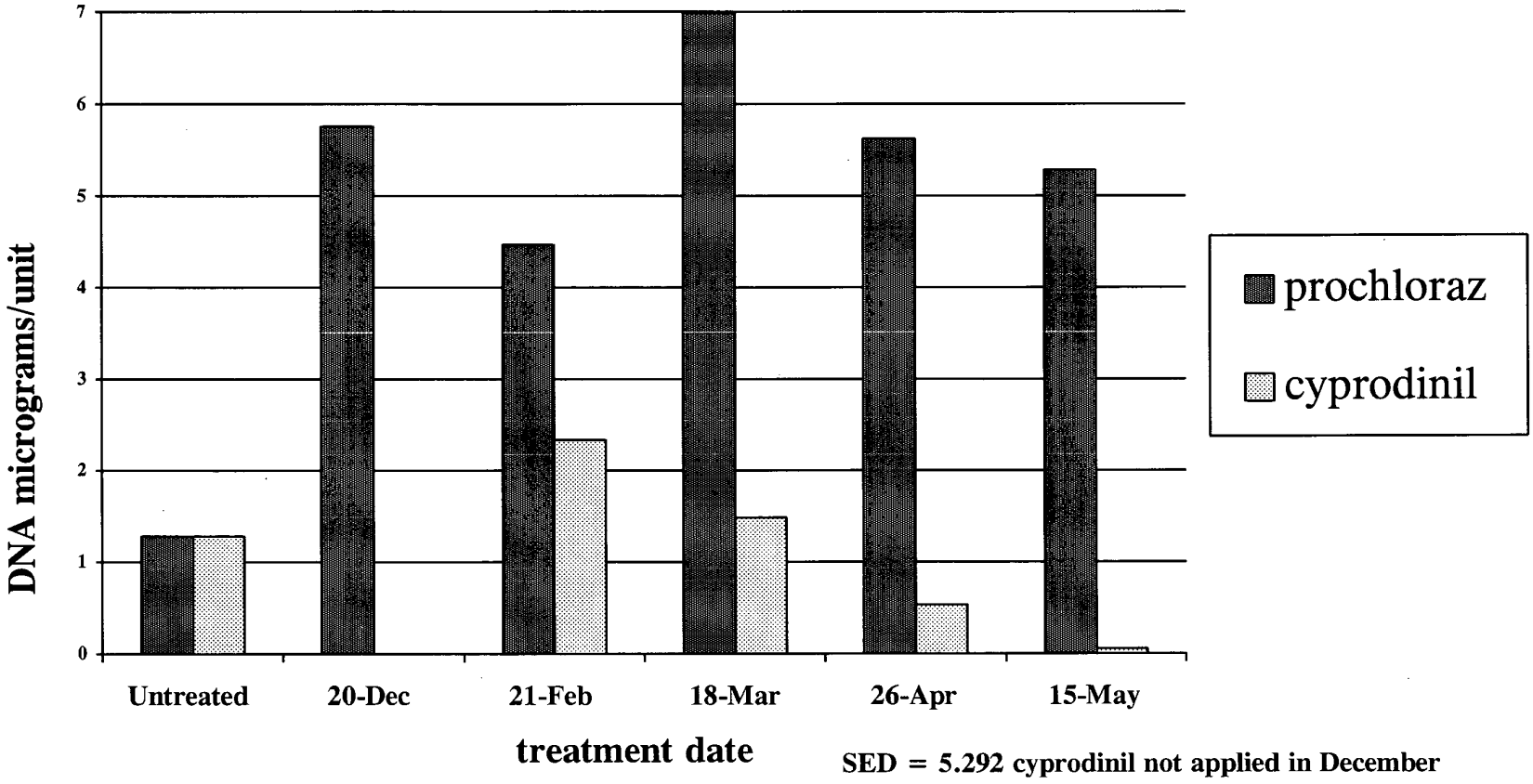


Figure 12.
W strain eyespot 1996 at GS 85



SED = 0.324 cyprodinil not applied in December

Figure 13.
R strain eyespot 1996 at GS 85



SED = 5.292 cyprodinil not applied in December

Figure 14.
Development of W strains 1996

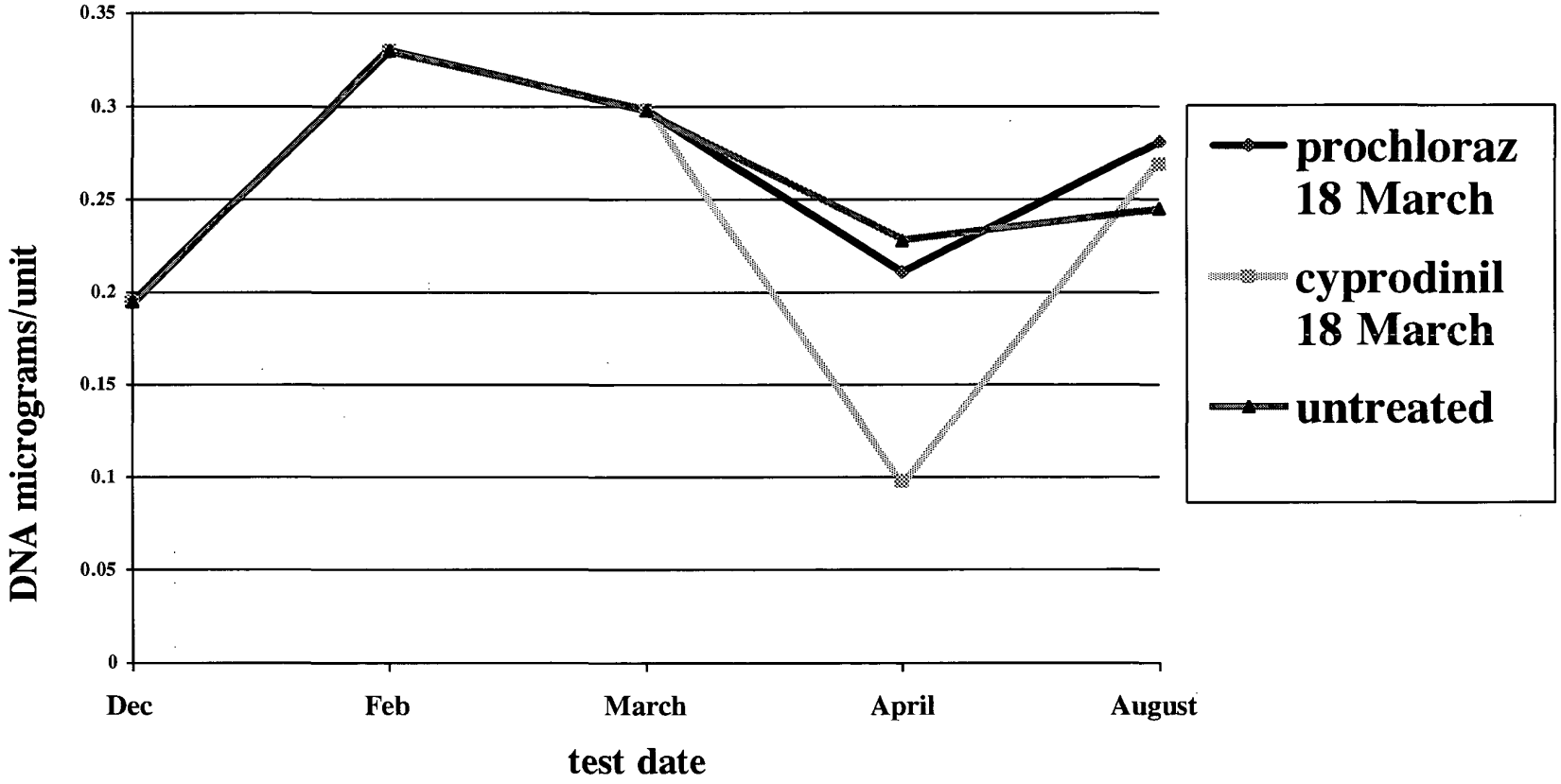


Figure 15.
Development of R strains 1996

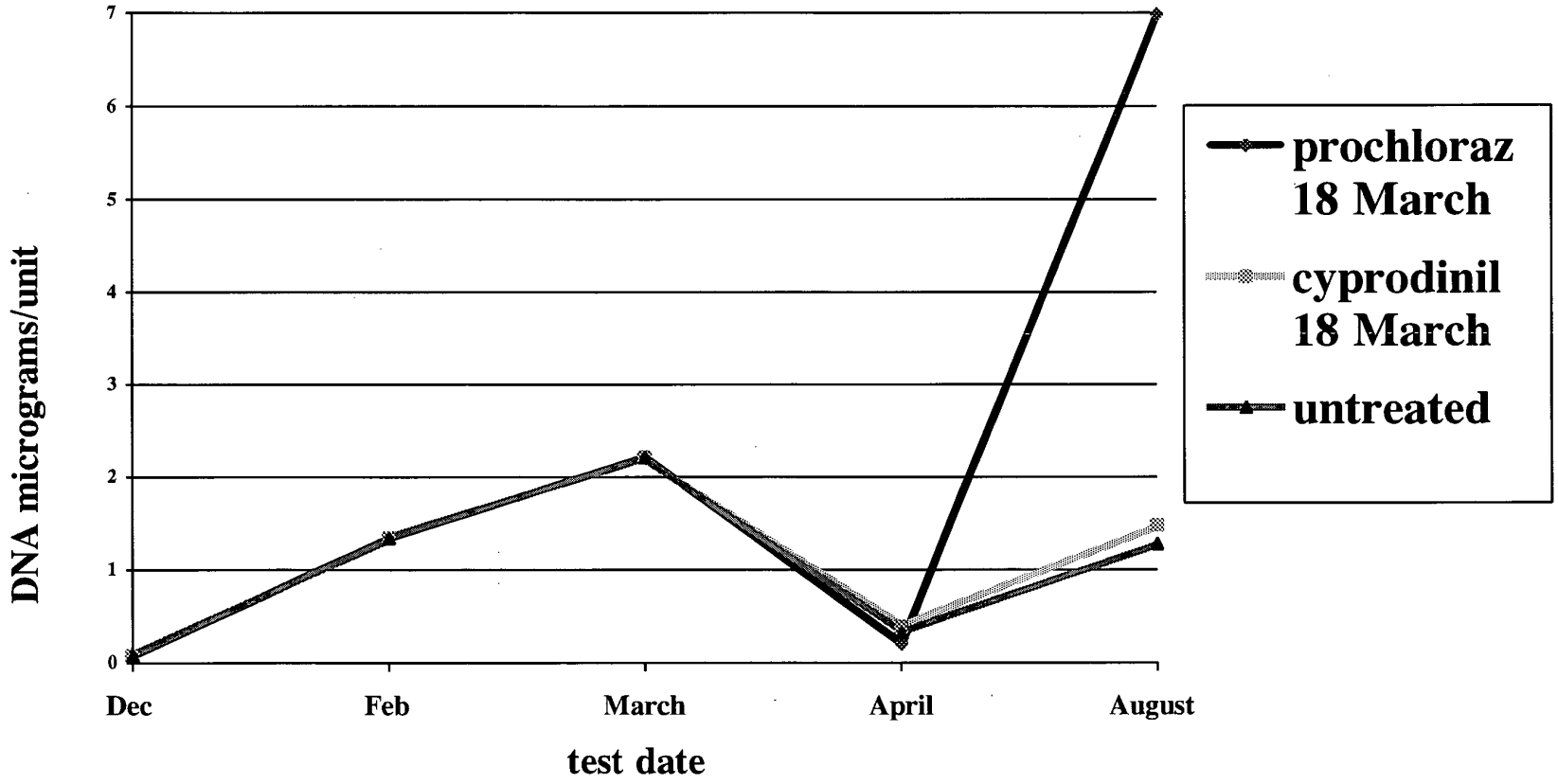


Figure 16.
Development of W strains 1996

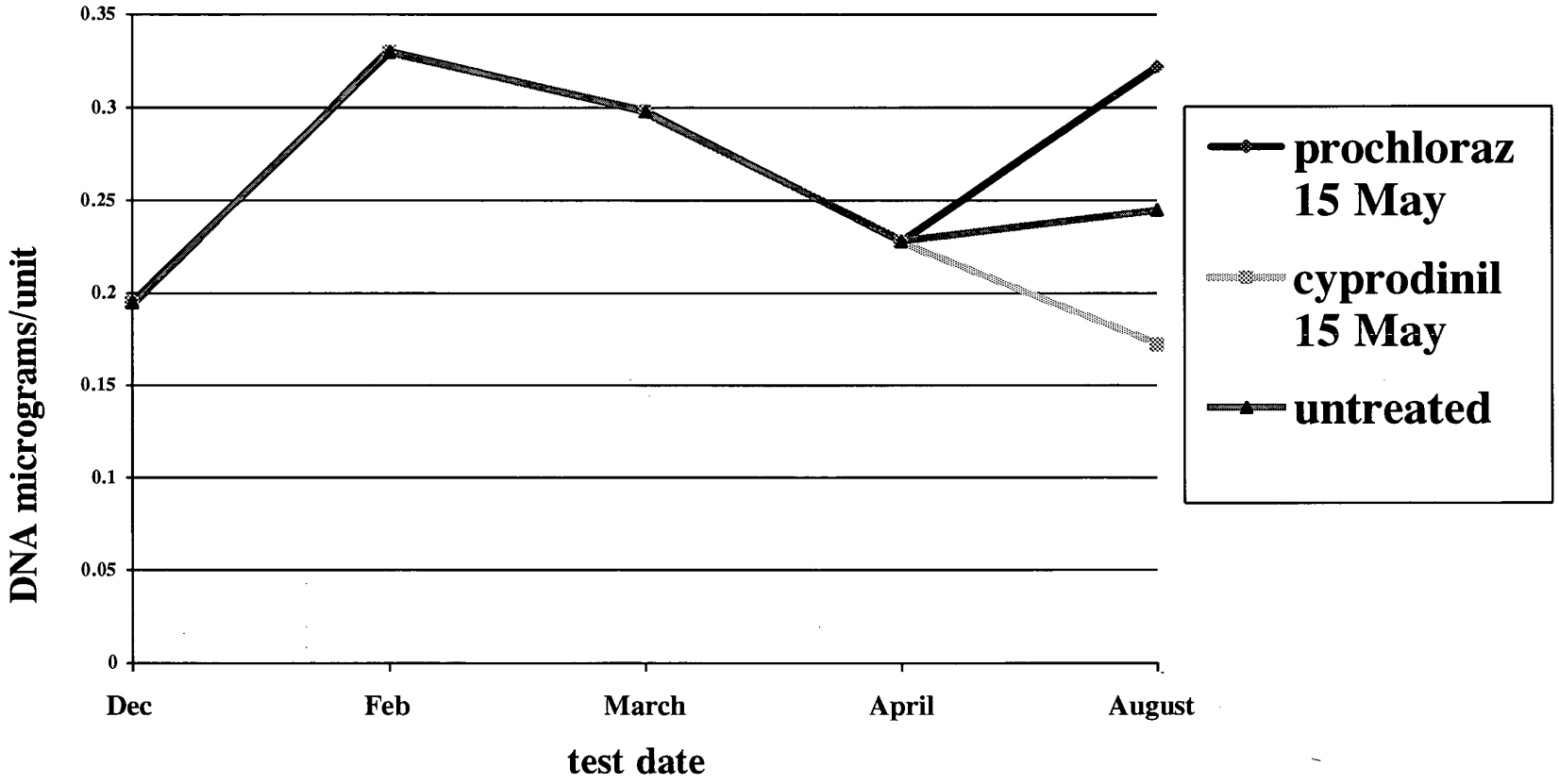
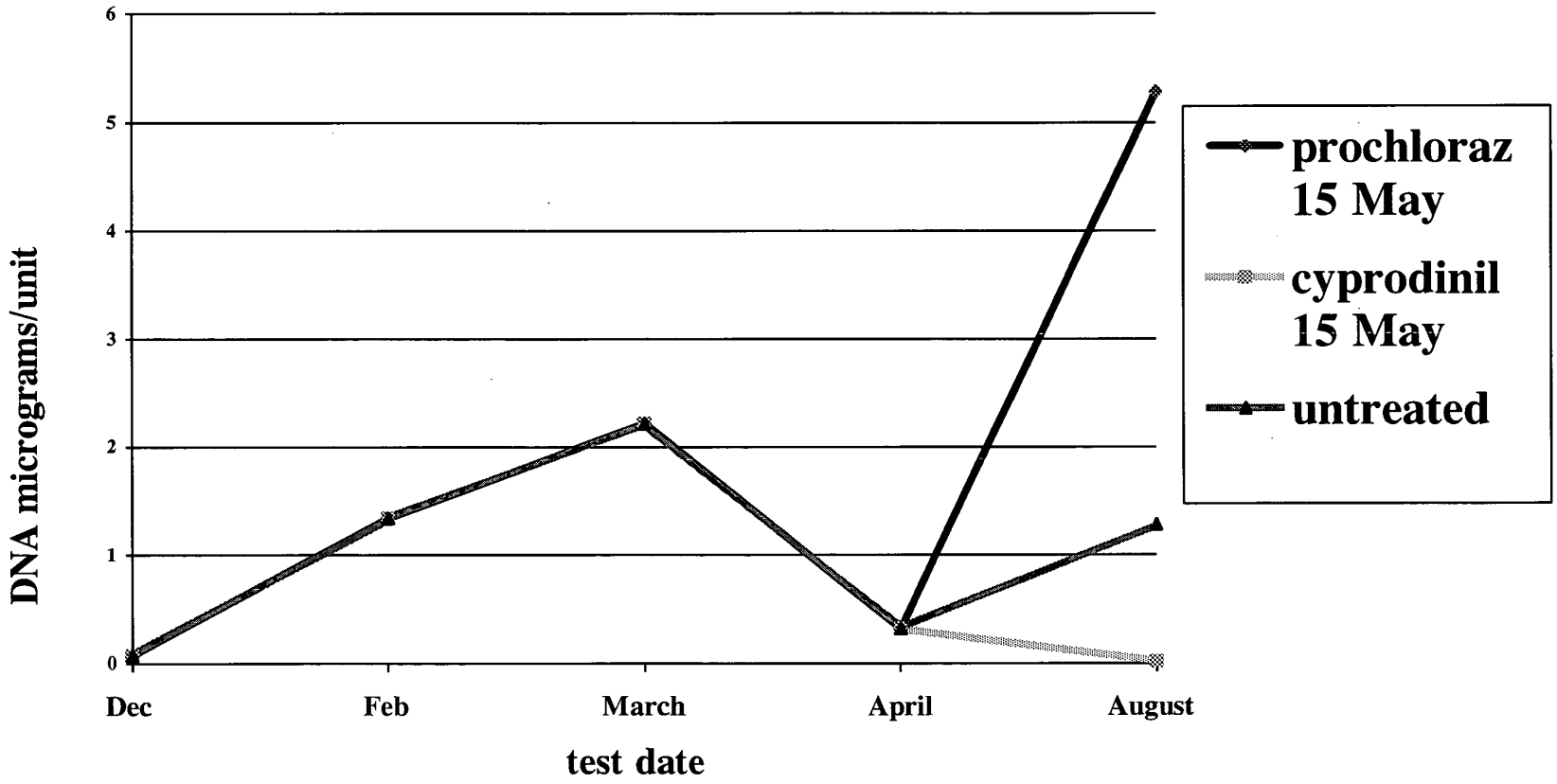


Figure 17.
Development of R strains 1996



4. DISCUSSION

Eyespot fungus and PCR techniques

The potential of the PCR-based technique as a tool for understanding the development of eyespot epidemics was demonstrated in the two years of trials. Using the technique it was possible to plot the development of the R and W strains and the dominance of one strain over another. The R strain predominated in both seasons trials in this work. The two strains tended to increase and decline together. Levels of both peaked at the start of stem extension and then declined during stem extension, before the levels determined at the end of the season were achieved. This decline at stem extension is probably as a result of the shedding of the outer leaves and accompanying lesions which have not infected the stem base, and similar dips have been noted in visual assessment throughout crop development. The project did not identify any competitive effect between the two eyespot strains, but the R strain was dominant at every stage so that even where it was reduced the W strain did not appear to take over. While there was no indication of a competitive effect between the R and W strain the 1995 data, where other stem base pathogens were also assessed with PCR, did show that cyprodinil had a controlling effect on sharp eyespot, and both fungicides reduced levels of *Microdochium nivale* early in the season (Appendix 2). The cleaning up of the stem base could have made it easier for the eyespot population to recover.

An important observation was that in 1996 the levels of eyespot DNA detected at the end of the season in the untreated plots were lower than in the treated plots. Visually the untreated plots had the most severe eyespot lesions. It is likely that a severely damaged and diseased stem cannot support the same levels of eyespot as a healthier, cleaner stem and so levels of eyespot decline in heavily diseased stems at the end of the season. The passage of water and nutrients through the stem is reduced where disease symptoms are high - and poor grain filling is the most obvious symptom of this. This reduction in the passage of water and nutrients may also make the severely diseased stem a less than ideal host for the eyespot fungus and hence while visual symptoms remain severe and cause significant yield loss in the host plant, the eyespot fungus itself may also be in decline. Eyespot symptoms are more severe when water is plentiful than in plants that are water-stressed (Higgins & Fitt, 1985), and a lack of water restricts the growth of the pathogen. This would suggest that PCR diagnostics may be used ahead of visual symptoms developing, but thereafter should always be used in conjunction with the severity of the visual symptoms. In this work there was no statistical evidence of a negative association between visual eyespot symptoms at the end of the season and levels of the eyespot fungus as measured using PCR, but there was certainly a decline in the association between PCR measured DNA and visual symptoms from that noted early in the season. Future work should study the levels of DNA from stem extension through to the end of the season. In this work no assessments of fungal DNA levels were made between the early stages of stem assessment and the final assessment at the end of the season. This period in the season is critical if we are to understand the progress of the eyespot pathogen in diseased and healthy stems and in order to accurately interpret DNA levels at the end of the season. From this work we can only assume that eyespot levels decline from stem extension to the end of the season in untreated plots whereas what is more likely is that they continue to increase to a point in the season where the stem becomes too damaged to support the eyespot and the levels of eyespot decline from this point until the end of the season. This has been demonstrated in an alternative host / pathogen situation where Mahuku *et al.* (1995)

demonstrated that DNA levels of *Leptosphaeria maculans* fell as the visible lesion size increased on oilseed rape, and the plant started to senesce. Before this point in symptom development DNA levels had been a good indicator of the amount of inoculum placed on the plants, an observation that may parallel what is occurring in the wheat / eyespot scenario.

An alternative explanation of the fall in DNA levels observed at the end of the season despite the fact that eyespot lesions were visible, would be that colonisation by other stem base pathogens was causing visible symptoms in a stem where eyespot levels were reduced by treatment. *Microdochium nivale* and R eyespot for example are found associated more often than would be expected by chance (P. Nicholson, per. comm.), evidence that interactions do exist between the pathogens of the stem base. In 1995 where DNA levels of the other stem base pathogens were determined there was no statistical evidence of any positive or negative interactions (Appendix 2).

The levels of DNA measured were variable between plots even within treatments and the differences between treatments were only occasionally significant. Eyespot is patchily, rather than evenly, distributed in fields (N. McRoberts, pers. comm.) and the variation in the PCR results is probably a factor of the sampling required to reduce variation between plots. Further study of how eyespot infection is distributed would be possible using PCR probes, and would allow for more accurate sampling for both visual and PCR based assessments.

The extraction procedure for the DNA has been modified so that results can now be quantified as a finite amount of fungal DNA rather than as an amount per unit of host tissue, the best available method when this work was carried out. This will take out one element of variation in the PCR results.

Disease control

Prochloraz consistently out-performed flusilazole in earlier work, but cyprodinil tested for the first time in 1994 gave comparable disease control to prochloraz, and also showed a significant yield benefit over the untreated. Cyprodinil and prochloraz were then evaluated in two years of trials at the East Lothian site, and the progress of the eyespot epidemics was followed by quantifying the amount eyespot DNA present at each assessment timing, as well as by making visual assessments of the eyespot present. The trials demonstrated that eyespot control and yield benefits can be achieved with cyprodinil and prochloraz sprays.

The variability of the PCR results meant that differences between treatments were seldom significant but several trends emerged over the two seasons. In general the PCR assessments showed that treatment with either prochloraz or cyprodinil gave a reduction in fungal DNA levels measured at the next assessment four weeks later, although levels always rose again after an initial reduction. Prochloraz for example gave a reduction in R strain DNA levels in 1996 that was still significantly less than the levels in the untreated plots three months after treatment. By following the increase in fungal DNA after treatment it was apparent that very often the populations could recover so that by the end of the season they had often exceeded the levels in the untreated plots. As mentioned above the levels of fungal DNA measured at the end of the season could be lower as a result of plant cell death at the stem base, and the PCR assessment at this time should be interpreted with the visual symptoms present. These show that treatment with prochloraz gave best control when applied early, at mid to late

tillering and cyprodinil did better the later it was used - the latest timing evaluated in this work was third node stage.

Assessments of the fungal DNA present were not made between stem extension and the ripening stages of crop growth and with more resources it would be possible to plot the development of the two strains during this critical phase. This could be the key to understanding which treatments are successful and which are not.

The amount of fungal DNA assessed after fungicide application demonstrated that both fungicides were more effective at controlling the W strain, than the R strain which was often able to recover from early control and result in severe infection late in the season. The greater efficacy of the two fungicides against the W strain in this study concurs with reports in the literature. Prochloraz has been reported to control the W strain better than the R strain (Bateman *et al.*, 1986) and cyprodinil showed better control of the W strain in work carried out in France (Migeon *et al.*, 1995).

There were differences in how the two strains responded to the different fungicides; control of the W strain with prochloraz was not as good after GS 31 as it was earlier in the season. Control of the W strain with cyprodinil was initially better than with prochloraz but the population often recovered faster after early treatments and either exceeded or matched the levels after prochloraz treatment. The optimum timing for W strain control with cyprodinil was therefore later in the season.

Cyprodinil gave a more persistent reduction in R strain eyespot in both seasons than prochloraz and although control of the R strain with prochloraz was initially good the population often recovered. Recovery of the R strain population was slower after cyprodinil treatment in both seasons. The PCR technology demonstrated that fungicide treatments work by reducing the levels of both strains present. Control was temporary, and the populations recovered, so the key to effectively reducing the degree of visual symptoms and the damage to the plant at the end of the season, is timing the fungicide application to achieve the longest respite from the disease possible.

Treatment too early or too late could allow the populations to recover, and visual eyespot symptoms to develop to severe levels despite the treatment. Prochloraz applied too early led to a recovery of the W population that eventually exceeded the levels in the untreated controls, but control of the R strain had to be made early with prochloraz. Application too late did not significantly reduce the R strain eyespot levels after application. Cyprodinil could give large reductions in R and W strain eyespot, but the populations could recover fast, particularly the W strain, so again it was evident that cyprodinil used late could reduce the populations over the remainder of the season.

Control of eyespot therefore remains a compromise between targeting the site of infection at early stem extension where this part of the plant is still exposed, but not going in so early that the eyespot populations can recover and eventually exceed the initial disease prognosis. The two fungicides may act differentially on the primary and secondary sources of inoculum, so that cyprodinil is somehow better able to control the eyespot that develops on the stem after the initial lesions on the outer leaf sheaths are shed. Prochloraz is known to act best as a

protectant and to have only limited curative activity against eyespot (Daniels (1993 b). It therefore has most effect by reducing the level of eyespot on the outer leaves and hence initial levels of inoculum. The physical targeting of prochloraz at the infection site is also known to be important in effective treatment (Cooke *et al.*, 1989). At the early timings cyprodinil gave large reductions in W and R strains that did not always persist to the end of the season. The reduction in eyespot DNA levels achieved with cyprodinil sprays later in the season, after stem extension, compared to sprays applied before this timing did persist and were also translated into a reduction in visible symptoms.

The way forward is therefore to examine the efficacy of sequences of fungicides so that these early reductions can be followed up later in the season with additional fungicide treatments so that the population is again reduced. It may be possible to utilise the finding that prochloraz is most effective early in the season, and cyprodinil late when evaluating sequences of fungicides. Future work is essential to confirm the trends that were seen in these studies and to reduce the variation in the PCR results. In particular it will be critical to determine how untreated populations develop in the later part of the season so that the apparent anomaly, observed in these studies where the untreated plots had low levels of eyespot DNA while visually they were the most diseased, can be explained. This could be a measure of the inaccuracy of visual assessment or it could be that visually the necrosis and cell browning around an eyespot lesion leads to a high eyespot score being given, but while this cell browning and death could be as damaging to the plant as the actual infection, it could also be a very impoverished host for the eyespot pathogen which may have declined from an earlier peak that caused the severe symptoms. Future investigation is warranted to clarify that this is not an anomaly in the PCR system. The findings of this work are that PCR assessment should be interpreted with visual symptoms after symptom development.

Thresholds

Visual assessment of eyespot carried out at stem extension did not correlate with the levels assessed visually at the end of the season, and the amount of eyespot DNA detected at stem extension also did not correlate with the levels detected at the end of the season. Levels of R strain eyespot detected by PCR at stem extension did not correlate with the levels found at the end of the season in 1996 and this was true for W strain eyespot in this season. In 1995 the levels of R strain DNA at stem extension were weakly associated with the levels detected at the end of the season, and there was a slightly stronger association between W strain DNA at stem extension and at the end of the season.

The significant association in 1995 between the W levels of DNA at stem extension (GS 31) and the visual symptoms recorded at the end of the season could indicate why in the past thresholds could be used more successfully than today. If the W strain had been the only strain present, levels of W strain DNA at stem extension would have been a good indicator of the risk of severe symptom development by the end of the season. Thresholds may therefore have been more successful when the W strain dominated in the UK, which was when thresholds for treatment were devised (Anon, 1987). W strain levels at stem extension in 1996 were not good indicators of the risk of severe symptom development - stem extension occurred a month earlier in this season which could have been a factor in why even W strain thresholds did not work in 1996.

In both seasons of these trials therefore total DNA at stem extension and at the end of the season did not correlate. This would make any attempt at determining a threshold at stem extension and before impossible, where a mixed population is present, and it would appear that even early prediction of the W levels in an un mixed population would often be unsuccessful.

5. CONCLUSIONS

Eyespot control and yield benefits can be achieved with cyprodinil and prochloraz sprays. Prochloraz has to be used early in the season, during tillering, for maximum effect on eyespot levels and cyprodinil after the start of stem extension. Spraying outside the optimum window could allow the eyespot populations to recover following treatment even if initial reductions were achieved. Prochloraz applied too late could not reduce the eyespot population sufficiently to affect the levels at the end of the season. In contrast cyprodinil applied too early achieved an initial reduction that could not be maintained until the end of the season. The findings of the work would suggest the potential for using sequences of fungicides to achieve season long control of the eyespot pathogen.

Both fungicides were more effective at controlling the W strain. Cyprodinil gave a more persistent reduction in R strain eyespot than prochloraz, and control of the R strain with prochloraz was initially good but the population often recovered. The optimum timing for W strain control with prochloraz was from mid-tillering to GS 31. The W population could recover fast after control with cyprodinil so that cyprodinil was better applied as late as possible.

PCR analysis showed that the R strain predominated in both seasons but both strains declined naturally at stem extension as old leaves were shed. These was no indication of a competitive effect between the two strains, but W levels were very low in comparison. PCR and visual assessments up to stem extension were not useful in determining eyespot levels at the end of the season so thresholds for treatment would not have worked.

This project highlighted the use of PCR diagnostics as a tool for understanding why treatments are effective. Using the technique it was possible to chart the initial efficacy of the fungicides following application, and the duration of this control. The work demonstrated that where reductions in eyespot levels, measured as the amount of fungal DNA present, were large and persistent enough, eyespot levels at the end of the season were reduced. The technique demonstrated that the eyespot population was able to recover following treatment so that the most successful treatments at reducing eyespot levels at the end of the season were those that had not only reduced eyespot levels initially, but also had been able to maintain this reduction for two or three months. Although in every case eyespot levels increased following the initial reduction after fungicide application, levels of eyespot DNA at the end of the season were sometimes still lower than in the untreated controls. Where this was not the case and DNA levels were not lower at the end of the season, a reduction in the level of visual symptoms and yield benefits could be demonstrated for those fungicide treatments where the initial reduction in DNA levels following treatment had been large enough and persistent enough. The work would also indicate that after symptom development PCR assessments should always be interpreted with the visual symptom data, as eyespot DNA levels eventually declined in necrotic plant tissue, such as was found at the end of the season following severe eyespot infection.

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APPENDIX ONE : CROP PROTECTION IN NORTHERN BRITAIN PAPER

THE IMPORTANCE AND CONTROL OF COMMON EYESPOT (PSEUDOCERCOSPORELLA HERPOTRICHOIDES) IN WHEAT

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Summary: Field trials were carried out over four seasons at a site in East Lothian to re-examine the timing of fungicide applications in Scottish winter wheat crops to control common eyespot (Pseudocercosporella herpotrichoides). Multiple fungicide sprays significantly reduced the levels of eyespot and lodging in the crops at the end of the season. Split or single fungicide applications at the mid to late tillering growth stages of the crops gave superior eyespot control when compared to the conventional GS 30 and GS 32 timings. Lodging control was also superior at these mid tillering timings. There was a strong association between eyespot levels and yield and a weaker correlation between degree of lodging and yield and eyespot and lodging. Two fungicides were evaluated, prochloraz and flusilazole. Prochloraz provided superior eyespot control and also gave the most effective reduction in lodging.

INTRODUCTION

The severity of disease development as a result of infection by eyespot (Pseudocercosporella herpotrichoides) is determined by cultural as well as agronomic factors, and is greatest under cool, moist conditions and where wheat and / or barley is grown in close rotation. Eyespot is conventionally controlled in winter wheat crops in Scotland with a fungicide spray at early stem extension (growth stages 30 to 32), often applied as a split treatment.

The yield loss associated with eyespot infection and its impact on crop lodging is unclear. Sutherland & Oxley (1993) found that control of eyespot at GS 31 did not always result in an increase in yield. Clarkson (1981) found a correlation between eyespot severity and individual plant yield loss. Scott & Hollins (1978) also showed a relationship between eyespot and yield, but reported that the correlation between yield loss and lodging was stronger.

The aim of this work was to examine the impact of eyespot infection on lodging and yield at a Scottish site, and to re-evaluate the spray timings for control of the disease.

MATERIALS AND METHODS

Trials were conducted on winter wheat over four seasons from 1991/1992 to 1994/1995. A series of four, three, two and single spray programmes of prochloraz or flusilazole at approximately fortnightly intervals during tillering and stem extension were evaluated in 1991/1992 and 1992/1993. In 1993/1994, in response to the results in the preceding seasons, the programme of prochloraz sprays was extended to include a later spray timing and extra split timings. The programme in 1994/1995 was simplified and prochloraz sprays were applied singly, at monthly intervals, and included a much earlier timing (Table 1).

The variety Beaver was sown in each season apart from the 1994/1995 season when the variety was Riband. The trials were located in East Lothian and were all sited in commercial second wheats. Plots were approximately 40 m^2 and were laid out in randomised blocks. There were three replicates of the treatments in each season apart from the 1994/1995 season when treatments were replicated four times. Fungicide treatments were applied using a hand held Cooper Pegler CP3 sprayer calibrated to deliver a water volume of 200 l/ha at a

pressure of 2.5 bars. All plots including the untreated were over sprayed with fungicides at GS 39 and GS 55 (Tottman and Broad, 1987) to eliminate foliar disease development. Except for fungicides the trial areas received the same inputs as the surrounding commercial crop.

The incidence of eyespot at GS 30/31 was assessed in each season. The percentage of tillers with visual symptoms at GS 30/31 was low in each year, and never exceeded 11% incidence, although eyespot developed to significant levels later in each season. Twenty five tillers per plot were assessed between GS 75 and GS 83 for an eyespot severity index, (number of slightly infected stems plus the number of moderately infected stems multiplied by two plus the number of severely infected stems multiplied by three) expressed as a percentage. Lodging (percentage of each plot leaning at more than 45°) and yield (tonnes per hectare corrected to 85% moisture content) were assessed at harvest. The treatment dates and crop growth stages are detailed in Table 1 and the spray programmes evaluated, in Table 2.

Table 1

Treatment spray dates and crop growth stages in each season

Target spray date	1992 Spray dates	1992 Growth stages	1993 Spray dates	1993 Growth stages	1994 Spray dates	1994 Growth stages	1995 Spray dates	1995 Growth stages
12/12	-	-	-	-	-	-	5/12	13,21
12/2	11/2	21,22	11/2	13/23	8/2	12,22	14/2	21,22
26/2	25/3	21,22	5/3	21,23	22/2	21,22		
11/3	11/3	23,25	11/3	22,23	8/3	23,25	14/3	25,27
25/3	24/3	25,27	25/3	23,25	22/3	25,27		
8/4	8/4	27,28	8/4	23,27	5/4	27,28	14/4	30
22/4	22/4	30,31	20/4	30,31	19/5	30,31		
6/5	5/5	31,32	5/5	33	3/5	31,32	1/5	32
17/5					17/5	33,37		

Table 2

Eyespot spray programmes evaluated on winter wheat trials

Target spray date	12/12	12/2	26/2	11/3	25/3	8/4	22/4	6/5	17/5	Product dose
T1	-	-	-	-	-	-	-	-	-	-
T2	-	P	-	P	-	P	-	P	-	½
T3	-	-	P	-	P	-	P	-	P	½
T4	-	-	P	-	P	-	P	-	-	½
T5	-	-	-	P	-	P	-	P	-	½
T6	-	-	-	-	P	-	P	-	P	½
T7	-	P	-	P	-	-	-	-	-	½
T8	-	-	P	-	P	-	-	-	-	½
T9	-	-	-	P	-	P	-	-	-	½
T10	-	-	-	-	P	-	P	-	-	½
T11	-	-	-	-	-	P	-	P	-	½
T12	-	-	-	-	-	-	P	-	P	½
T13	-	P	-	-	-	-	P	-	-	½
T14	-	P	-	-	-	-	-	-	P	½
T15	P	-	-	-	-	-	-	-	-	*f*
T16	-	P	-	-	-	-	-	-	-	*f*
T17	-	-	P	-	-	-	-	-	-	*f*
T18	-	-	-	P	-	-	-	-	-	*f*
T19	-	-	-	-	P	-	-	-	-	*f*
T20	-	-	-	-	-	P	-	-	-	*f*
T21	-	-	-	-	-	-	P	-	-	*f*
T22	-	-	-	-	-	-	-	P	-	*f*
T23	-	-	-	-	-	-	-	-	P	*f*
T24	-	F	-	F	-	F	-	F	-	½
T25	-	-	F	-	F	-	F	-	-	½
T26	-	-	-	F	-	F	-	F	-	½
T27	-	-	-	-	F	-	F	-	-	½
T28	-	-	-	-	-	F	-	F	-	½
T29	-	F	-	-	-	-	-	-	-	*f*
T30	-	-	F	-	-	-	-	-	-	*f*
T31	-	-	-	F	-	-	-	-	-	*f*
T32	-	-	-	-	F	-	-	-	-	*f*
T33	-	-	-	-	-	F	-	-	-	*f*
T34	-	-	-	-	-	-	F	-	-	*f*
T35	-	-	-	-	-	-	-	F	-	*f*
T36	-	-	-	-	-	-	-	-	-	-

½ = half commercial dose *f* = full commercial dose

Full commercial doses for the products used were as follows:-

Active ingredient	*Product*	*Manufacturer*	*g a.i./ha*
P = prochloraz	Sportak 45	AgrEvo	405
F = flusilazole	Sanction	Du Pont	200

RESULTS

The results from each season were meaned and are presented in Tables 3 (multiple sprays) and 4 (single sprays). Multiple four or three spray programmes of prochloraz (T2 - T6) significantly reduced the levels of eyespot and lodging, and improved yield (Table 3) compared to the untreated (T1 and T36). T3 was evaluated in one season only. A two spray programme encompassing the conventional stem extension timings (T11 applied at GS 27/28 and 31/32) provided significant eyespot and lodging control. Control of both had declined by the T12 timings (GS 30/31 and 33/37). A similar programme applied at the mid tillering timings (T10 applied at GS 25/27 and 30/31), however, gave superior control of both eyespot and lodging. In comparison with prochloraz, flusilazole showed a reduced level of control. The conventionally timed split spray (T28) did not give significant eyespot or lodging control, but a split treatment at mid tillering (T27) did, and also significantly improved yield. Flusilazole also performed best for lodging control at the mid tillering timing (T27) but the decline in control by late tillering (T28) was more marked than for prochloraz (T11).

Table 3

Effect of multiple sprays on eyespot lodging and yield.

Treatment	Eyespot index %	Lodging %	Yield t/ha	Treatment	Eyespot index %	Lodging %	Yield t/ha
T1	47.6	36.9	9.41	T1	47.6	36.9	9.41
T2	10.12	0	10.38	T24	28.04	29.1	9.92
T3	24.26	16.8	10.09				
T4	8.26	0	10.23	T25	31.53	2.5	10.14
T5	19.74	4.0	10.16	T26	39.68	20.8	9.78
T6	27.37	16.8	10.55				
T7	27.82	16.8	9.74				
T8	24.26	16.8	9.98				
T9	34.04	16.8	9.78				
T10	15.79	5.0	10.02	T27	33.38	8.8	9.91
T11	27.50	6.1	9.98	T28	46.33	33.3	9.69
T12	29.15	16.8	9.81				
T13	26.04	16.8	9.22				
T14	31.37	15.8	10.03				
T36	47.94	32.8	9.31	T36	47.94	32.8	9.31
LSD (P≤0.05)	9.478	15.58	0.344	LSD (P≤0.05)	9.478	15.58	0.344

For the multiple prochloraz sprays there was a strong negative correlation between eyespot and yield ($r = -0.817$, $P \leq 0.001$). The correlation between eyespot and lodging was not so strong ($r = 0.603$, $P \leq 0.005$). Lodging was not as closely associated with yield ($r = -0.448$, $P \leq 0.02$). The association between yield and eyespot was even closer for the flusilazole treatments ($r = -0.978$, $P \leq 0.001$). Lodging and yield and eyespot and lodging were less closely associated ($r = -0.760$, $P \leq 0.01$ and 0.603, $P \leq 0.05$ respectively).

Single spray timings showed that a single spray of prochloraz at the very early stages of tillering (T15 and 16 applied at GS 13/21 and GS 21/22) gave the best control of eyespot (Table 4) compared to the untreated T1 and T36. Lodging was also significantly reduced at this timing, but the reduction in lodging was superior at a mid tillering timing (T19, GS 25/27). Sprays at the start of stem extension did not significantly reduce lodging (T21 and T22, GS 30/31 and GS 31/32 respectively). T23 (GS 33/37) was evaluated in one season only. Split sprays T13 and T14 (Table 3) were included in the 1993/1994 season to see if it was possible to combine the eyespot control at early tillering with improved lodging control at mid tillering, but did not show a yield benefit over other split treatments. Single sprays of flusilazole did not significantly reduce eyespot or lodging, or improve yield at these timings, although there was a tendency for lodging control to be best at the mid tillering timings and to decrease by stem extension.

Table 4

Effect of single sprays on eyespot, lodging and yield.

Treatment	Eyespot index %	Lodging %	Yield t/ha	Treatment	Eyespot index %	Lodging %	Yield t/ha
T1	47.6	36.9	9.41	T1	47.6	36.9	9.41
T15	30.87	16.8	9.97				
T16	30.5	16.1	9.79	T29	42.49	38.3	9.03
T17	32.68	16.1	9.62	T30	46.46	26.1	9.62
T18	41.03	21.6	9.76	T31	42.57	21.6	9.57
T19	31.10	4.5	9.82	T32	37.87	20.0	9.70
T20	32.53	17.0	10.13	T33	42.04	20.0	9.76
T21	33.74	20.9	10.96	T34	39.64	21.1	9.68
T22	34.01	18.7	9.84	T35	39.96	34.1	9.60
T23	31.82	16.8	10.43				
T36	47.94	32.8	9.31	T36	47.94	32.8	9.31
LSD (P≤0.05)	9.478	15.58	0.344	LSD (P≤0.05)	9.478	15.58	0.344

For the single prochloraz and flusilazole sprays there was a very strong negative correlation between eyespot and yield ($r = -0.907$, $P \leq 0.001$ and -0.944, $P \leq 0.001$ respectively). The correlation between eyespot and lodging not so strong, $r = 0.603$, $P \leq 0.001$ for the prochloraz treatments and $r = 0.603$, $P \leq 0.01$ for the flusilazole treatments. Lodging was not so closely associated with yield ($r = -0.583$, $P \leq 0.001$) for prochloraz, although there was a slightly stronger association for the flusilazole treatments ($r = -0.722$, $P \leq 0.001$).

DISCUSSION

The results of these trials showed that there was a significant association between eyespot levels and yield. Although lodging was also shown to be associated with yield loss, the correlation was not as strong as that between eyespot and yield. There was also a significant correlation between eyespot and lodging.

By the conventional timing of stem extension the optimum timing for eyespot control had already passed for both split treatments and single sprays. Flusilazole was generally poorer than prochloraz for both eyespot and lodging control, but the pattern of control was similar in that mid tillering was superior in terms of eyespot and lodging control for the split treatments evaluated. The window for lodging control appeared to be narrower and control declined more rapidly as stem extension approached for the flusilazole sprays. Griffin (1994) reports flusilazole to provide equivalent or superior control to prochloraz on many occasions. Flusilazole is most active against the W pathotype in the eyespot population, however testing of the pathotypes at the sites used in this work showed the populations were predominantly R type.

Single full dose sprays of prochloraz applied at GS 13 to GS 22 as early as December gave surprisingly good control of eyespot as well as significantly greater yield. While this may not represent a cost-effective option in terms of application costs there is perhaps potential for a seed treatment with activity at this time. Griffin (1994) also reports superior control from early sprays as compared to later sprays at English sites where significant eyespot symptoms developed and foliar diseases were not a complicating factor. The success of early treatments could be due to successful targeting of infection sites prior to stem extension.

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APPENDIX TWO: DATA TABLES

1994 Assessments

Table 1. Eyespot incidence weeks 1- 7

	Wk1 NoD	Wk2 NoD	Wk3 NoD	Wk4 NoD	Wk5 NoD	Wk6 NoD	Wk7 NoD
T1	1.17	0	0.16	0.16	0.50	0.34	0.16
T2		0	0	0	0.33	0	0.33
T3				0	0.33	0.33	1.33
T4						1.00	0.67
T5		0	0	0.33	0	0	1.67
T6					1.33	1.00	0.67
T7						1.00	1.33
T8		0	0	0	0	0	0
T9				0.67	1.0	0.67	0.67
T10						0	1.00
SED		0.773	0.164	0.780	0.782	0.571	0.990

Table 2. 1994 Eyespot indexes weeks 8-11, with yield, thousand grain weight and specific weight.

	Wk8	Wk9	Wk10	Wk11	Yield	TGW	SpWt
T1	6.70	1.30	26.7	80.0	10.45	50.00	74.77
T2	4.00	5.30	14.7	46.7	10.35	50.83	74.73
T3	4.00	5.30	22.7	52.0	10.18	48.37	73.98
T4	5.30	8.00	12.0	52.0	10.72	50.23	74.63
T5	2.77	6.70	24.0	78.7	10.51	50.83	75.05
T6	8.00	4.00	33.3	65.3	10.27	50.37	74.50
T7	4.00	4.00	10.7	50.7	10.86	49.83	74.53
T8	2.70	5.30	10.7	52.0	10.51	50.57	74.25
T9	9.30	6.70	32.0	45.3	10.75	51.33	74.90
T10	2.70	6.70	21.3	57.3	10.50	51.33	74.87
SED	2.564	5.722	12.81	27.69	0.299	1.126	0.752

1995 Assessments

Table 3. 1995 Eyespot incidence weeks 1-4, eyespot index weeks 5-7

	Wk1 NoD	Wk2 NoD	Wk3 NoD	Wk4 NoD	Wk5 index	Wk6 index	Wk7 index
T1	0.00	0.50	1.5	0.75	3.00	18.33	49.67
T2	0.00	1.00	0.00	1.00	0.33	10.33	22.00
T3		2.00	1.00	1.25	1.00	10.33	17.67
T4			0.75	1.00	2.00	12.67	21.00
T5				0.75	1.33	7.00	25.67
T6					2.33	15.00	23.67
T7	0.00	0.50	0.25	0.75	2.33	13.67	35.67
T8		0.50	1.00	0.25	2.33	17.00	41.00
T9			1.00	0.75	1.00	17.00	44.00
T10				0.25	1.00	13.00	41.33
T11					1.00	6.33	31.00
SED		0.619	0.680	0.828	0.860	2.872	5.436

Table 4. 1995 Incidence of sharp eyespot weeks 1-4, eyespot index weeks 5-7

	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7
T1	0.00	0.00	0.00	0.00	0.33	4.00	2.67
T2	0.00	0.00	0.00	0.00	0.67	0.67	3.33
T3		0.25	0.00	0.00	1.67	5.00	2.67
T4			0.00	0.00	0.00	2.67	4.00
T5				0.00	1.33	12.33	1.33
T6					2.00	2.67	2.67
T7	0.00	0.00	0.00	0.25	1.00	1.33	2.00
T8		0.00	0.00	0.00	1.00	1.00	3.67
T9			0.00	0.00	1.67	5.67	4.00
T10				0.25	1.33	5.33	4.00
T11					1.00	1.33	3.33
SED		0.158		0.156	1.000	3.821	3.064

Table 5. 1995 Fusarium incidence weeks 1-4, eyespot index weeks 5-7

	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7
T1	0.50	1.75	12.25	13.00	16.00	21.00	35.67
T2	1.00	9.50	9.00	9.00	15.33	27.00	26.0
T3		12.75	7.50	14.00	14.33	21.67	31.33
T4			10.75	13.50	13.33	24.00	28.0
T5				9.25	16.33	16.33	32.0
T6					18.33	22.33	22.33
T7	1.25	13.75	8.00	14.00	15.33	28.00	21.0
T8		10.50	9.50	12.50	13.33	20.67	20.33
T9			10.00	13.75	7.00	18.67	30.0
T10				10.50	11.67	18.33	17.33
T11					15.67	11.00	17.33
SED	0.755	2.280	1.942	2.559	14.241	20.823	25.582

Table 6. 1995 lodging, greenleaf area and white heads

	Lod 1	Lod 2	Lod 3	Lod 4	Lod 5	GLA	WHITE
T1	20.02	15.75	25.00	31.25	37.50	36.25	37.50
T2	1.28	2.75	4.50	10.75	6.00	53.25	16.75
T3	0.52	3.75	4.75	9.75	6.50	52.50	30.00
T4	1.80	2.25	6.25	6.50	4.75	46.25	25.00
T5	1.52	5.25	7.00	8.00	4.75	36.75	32.75
T6	1.52	3.00	4.25	7.00	7.00	54.00	28.75
T7	4.25	6.00	15.5	26.25	22.50	35.75	14.50
T8	4.25	5.00	9.00	8.75	16.25	43.75	25.00
T9	8.62	6.00	10.00	10.00	13.25	32.50	39.25
T10	12.52	8.25	15.75	18.25	21.25	35.00	23.00
T11	20.00	13.00	20.00	20.00	27.00	51.25	45.00
SED	7.416	4.822	6.578	6.070	8.124	13.191	11.136

Lod 1 = Lodging 31/7/95
Lod 2 = Lodging 3/8/95
Lod 3 = Lodging 11/8/95
Lod 4 = Lodging 18/8/95
Lod 5 = Lodging 25/8/95

GLA = green leaf area31/7/95
WHITE = whiteheads 31/7/95

PCR assessments 1995

Table 7. PCR analysis of *Fusarium culmorum* 1995 (DNA µg/unit plant tissue)

Date	December	March	May	August
Growth stage	GS 13/21	GS 25/27	GS 32	GS 87
T1	0.00	0.00	0.00	0.75
T2	0.00	0.00	0.00	0.75
T3		0.00	0.00	1.25
T4		0.00	0.00	1.25
T5			0.00	1.33
T6			0.00	0.25
T7	0.00	0.00	0.00	0.25
T8		0.00	0.00	0.50
T9		0.00	0.00	1.50
T10			0.00	0.25
T11			0.00	1.00
P	*	*	*	0.475
SED	*	*	*	0.762

Table 8. PCR Analysis of *Fusarium avenacium* 1995 (DNA µg/unit plant tissue)

Date	December	March	May	August
Growth stage	GS 13/21	GS 25/27	GS 32	GS 87
T1	0.33	0.25	0.25	0.75
T2	0.00	0.25	0.25	0.00
T3		0.00	0.00	0.25
T4		0.00	0.00	0.50
T5			0.00	0.33
T6			0.50	0.00
T7	0.67	0.25	0.25	1.00
T8		0.00	0.00	0.75
T9		0.25	0.50	1.00
T10			0.25	0.50
T11			0.00	0.25
P	0.204	0.835	0.448	0.674
SED	0.356	0.316	0.318	0.672

Table 9. PCR Analysis of *Microdochium nivale* 1995 (DNA µg/unit plant tissue)

Date Growth stage	December GS 13/21	March GS 25/27	May GS 32	August GS 87
T1	2.00	2.00	3.00	1.50
T2	2.00	1.75	3.00	2.00
T3		2.25	3.00	2.25
T4		1.25	1.25	1.25
T5			2.25	1.33
T6			2.25	1.00
T7	2.00	2.00	2.25	1.25
T8		2.25	1.75	0.75
T9		1.25	2.25	0.75
T10			1.25	1.00
T11			2.50	0.75
P	*	0.390	0.046	0.069
SED	*	0.661	0.700	0.580

Table 10. PCR Analysis of Sharp eyespot 1995 (DNA µg/unit plant tissue)

Date Growth stage	December GS 13/21	March GS 25/27	May GS 32	August GS 87
T1	1.00	0.75	0.750	1.25
T2	2.50	0.25	1.00	0.25
T3		0.25	1.50	0.00
T4		0.00	0.25	0.25
T5			1.00	1.00
T6			1.50	0.50
T7	3.00	0.50	0.50	0.00
T8		0.50	1.00	0.00
T9		0.50	1.75	0.50
T10			0.75	0.00
T11			0.75	0.00
P	0.017	0.885	0.604	0.147
SED	0.535	0.649	0.813	0.297

1996 Assessments

Table 11. 1996 Eyespot Incidence Weeks 1- 3, Index Weeks 4 and 5

	Wk1	Wk2	Wk3	Wk4	Wk5
T1	3.00	2.25	1.25	1.67	26.08
T2	2.25	1.00	1.75	1.33	15.67
T3		2.25	1.00	2.00	10.67
T4			0.75	0.67	21.00
T5			1.25	1.67	19.33
T6					21.00
T7		3.00	2.25	1.33	15.33
T8		2.75	1.00	2.67	24.33
T9			2.25	0.67	26.00
T10			1.75	0.00	18.75
T11					17.00
SED	1.724	1.675	0.887	0.891	4.775

Table 12. 1996 Incidence of sharp eyespot Weeks 1-3, Index Weeks 4 and 5

	Wk1	Wk2	Wk3	Wk4	Wk5
T1	0.00	0.00	0.00	3.33	6.67
T2	0.00	0.00	0.25	3.67	8.00
T3		0.00	0.00	2.33	10.00
T4			0.00	3.00	9.33
T5			0.00	1.00	2.67
T6					6.67
T7		0.00	0.00	5.00	6.33
T8		0.00	0.00	3.33	4.00
T9			0.00	3.67	2.67
T10			0.00	2.00	4.33
T11					4.33
SED	*	*	0.036	1.849	2.398

Table 13. 1996 Fusarium incidence Weeks 1-3, Index Weeks 4 and 5

	Wk1	Wk2	Wk3	Wk4	Wk5
T1	8.25	14.75	8.50	21.33	15.67
T2	7.00	12.75	6.50	14.33	10.67
T3		12.00	7.50	16.00	21.00
T4			7.50	15.67	19.33
T5			8.00	20.33	21.00
T6					15.33
T7		10.25	9.00	12.33	24.33
T8		14.25	7.75	15.67	26.00
T9			7.00	10.67	18.75
T10			9.75	19.00	16.33
T11				19.67	17.00
SED	0.868	2.921	2.120	2.921	3.867

Table 14. 1996 FOLIAR ASSESSMENT AT GS 53

	MILDEW			SEPTORIA			GREEN LEAF AREA		
	leaf 1	leaf 2	leaf3	leaf1	leaf 2	leaf 3	leaf 1	leaf 2	leaf 3
1 A	0.00	0.22	1.18	0.00	0.25	0.32	100.0	100.0	100.0
2 B	0.00	0.02	0.55	0.00	0.08	0.22	100.0	100.0	99.5
3 C	0.00	0.10	0.32	0.00	0.00	0.25	100.0	99.7	100.0
4 D	0.00	0.05	0.98	0.00	0.02	0.12	100.0	100.0	99.2
5 E	0.00	0.02	0.48	0.00	0.05	0.12	98.8	100.0	98.1
6 F	0.00	0.05	0.89	0.00	0.05	0.20	100.0	100.0	100.0
7 G	0.00	0.08	0.35	0.00	0.00	0.18	98.6	100.0	100.0
8 H	0.00	0.00	0.40	0.00	0.58	0.12	100.0	100.0	100.0
9 I	0.00	0.05	0.80	0.00	0.05	0.22	100.0	99.0	100.0
10 J	0.00	0.05	0.65	0.00	0.00	0.25	100.0	100.0	100.0
11 K	0.00	0.08	0.97	0.00	0.02	0.12	100.0	100.0	100.0
12 L	0.00	0.02	0.40	0.00	0.05	0.20	100.0	100.0	100.0
13 M	0.00	0.08	0.50	0.00	0.00	0.25	100.0	100.0	100.0
14 N	0.00	0.05	0.60	0.00	0.02	0.22	100.0	100.0	100.0
15 0	0.00	0.12	0.75	0.00	0.02	0.18	100.0	100.0	100.0
16 P	0.00	0.10	0.72	0.00	0.05	0.27	100.0	100.0	100.0
SED	*	0.082	0.317	*	0.205	0.105	0.205	0.426	0.606

Table 15. 1996 FOLIAR ASSESSMENT AT GS 73

	MILDEW			SEPTORIA			GREEN LEAF AREA		
	leaf 1	leaf 2	leaf3	leafl	leaf 2	leaf 3	leaf 1	leaf 2	leaf 3
1 A	2.60	3.72	10.75	6.75	14.75	0.80	84.2	70.0	3.2
2 B	1.28	2.28	5.0	4.30	12.00	2.18	85.2	51.5	23.5
3 C	1.88	2.62	18.5	4.30	14.15	4.52	85.5	49.2	17.4
4 D	2.15	2.32	5.25	6.50	12.25	5.00	82.4	58.2	5.0
5 E	1.35	3.35	6.50	4.75	20.50	0.62	84.8	66.0	6.0
6 F	2.18	3.82	3.85	8.38	14.55	3.20	85.2	60.0	4.8
7 G	1.25	3.65	6.25	7.02	16.95	5.00	84.6	46.8	4.2
8 H	2.10	1.42	4.0	3.28	10.75	1.20	86.8	67.0	7.0
9 I	1.52	5.58	6.25	5.72	7.98	0.62	87.0	75.2	4.2
10 J	2.38	3.18	7.25	2.62	13.75	4.38	70.8	54.5	5.0
11 K	1.40	2.52	5.50	5.25	13.50	2.80	84.8	71.0	9.4
12 L	1.38	1.82	14.25	5.70	12.50	3.05	85.8	66.5	19.1
13 M	1.65	2.05	3.40	5.55	9.50	2.78	87.5	48.0	5.2
14 N	1.40	1.28	5.75	6.00	8.50	2.08	83.2	50.8	8.5
15 0	2.08	2.88	5.75	5.25	15.75	3.50	86.8	47.8	5.2
16 P	1.65	2.08	9.75	5.30	6.75	2.30	86.8	72.5	5.2
SED	0.876	1.679	6.055	1.961	3.941	2.582	4.91	17.32	8.06

APPENDIX THREE: SITE DETAILS

Site details	**1995**				
Site Address	Markle Mains East Linton East Lothian	**Grid reference**	NT 558 771		
Field	**High Park East**				
Soil series	Brownrig	**Soil analysis**	pH	7.1	
			P index		9.9
			K index	217	
		Organic matter	7.0%		
Drainage	Under drained				
Previous cropping	1991 WW 1992 WW 1993 Peas 1994 WW	**Disposal of previous crop residue**	ploughed in		
Variety	Riband	**Date sown**	4/10/95		
Seed rate		**Plot size**	20m x 2m		

Treatments and date applied to experimental area

Fungicides	Silvacur 1.0 l/ha + Patrol 0.3 l/ha	14/6/95
Herbicides	Cougar 0.5 l/ha + IPU 0.75 l/ha Starane	25/10/94
Insecticides	none	
Growth regulators	none	
Fertiliser (Kg/ha)	218 N, 39 P, 39 K	
Harvest date	25/8/95	

Site details	**1996**			
Site Address	Markle Mains East Linton East Lothian	**Grid reference**	NT 558 771	
Field	**Below Road**			
Soil series	Brownrig	**Soil analysis**	pH P index K index Mg index	7.3 5.0 143 201
Drainage	Under drained			
Previous cropping	1993 Peas 1994 WW 1995 SOSR	**Disposal of previous crop residue**	ploughed in	
Variety	Riband	**Date sown**	25/09/96	
Seed rate	180 kg/ha	**Plot size**	20m x 2m	
Harvest date	25/8/95			

Treatments and date applied to experimental area

23/10/95	JAVELO	1.20 L/HA
23/10/95	CYPERMETHRIN	0.25 L/HA
29/04/96	CMPP	2.00 L/HA
29/04/96	CHLORMEQUAT	2.00 L/HA

FERTILISER FOR CURRENT CROP

Name of Fertiliser	Qty	Units	Stage of application
Hen Pen 39P 39K	11.0	KG/HA	PRE SOWING
30 N 18 S	4	KG/HA	27/02/
34.5% N	5.06	KG/HA	02/04/96
34.5% N	5.35	KG/HA	04/05/96

Total KG of fertiliser per HA 240 KG N, 39 KG P, 39 KG K