4. MATERIALS AND METHODS

The field trial was carried in 1998 by superimposing plots in a commercial crop of winter wheat (variety Riband) on a site in East Lothian. The crop was a second wheat. Plots were 40 m^2 and were laid out in randomised blocks. There were four replicates of the treatments. 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. The plots were not over sprayed with fungicides later in the season to eliminate foliar disease development. Except for fungicides the trial areas received the same inputs as the surrounding commercial crop.

Visual assessments for stem base diseases were carried out according to the four point scales below, on 25 separate plants from each plot (prior to growth stage 31) or tillers after this growth stage.

Score	Description
0	No symptoms
1	Lesions affecting less that 50% of stem circumference
2	Lesions affecting over 50% of stem circumference
3	Lesions affecting over 50% of stem circumference AND tissues softened so that lodging would readily occur.

A stem base disease percentage index was then calculated for each disease using the following

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

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

Table 1.
Spray programmes evaluated 1998
Treatment regimes 1 - 15

	GS 25 25 Feb 98	GS 30 21 April 98	GS 31 28 April 98	GS 32 11 May 98
1.	-	-	-	-
2.	Prochloraz 0.9 l/ha	-	-	-
3.	-	-	-	Cyprodinil 1.0 kg/ha
4.	-	Prochloraz 0.45 l/ha	-	Cyprodinil 0.5 kg/ha
5.	-	Cyprodinil 0.5 kg/ha	-	Prochloraz 0.45 l/ha
6.	Prochloraz 0.45 l/ha	-	Prochloraz 0.45 l/ha	-
7.	-	Cyprodinil 0.5 kg/ha	-	Cyprodinil 0.5 kg/ha
8.	Prochloraz 0.45 l/ha + Azoxystrobin 0.5 l/ha	-	-	-
9.	-	-	-	Cyprodinil 0.5 kg/ha + Azoxystrobin 0.5 l/ha
10.	Azoxystrobin 1.0 l/ha	-	-	-
11.	-	Azoxystrobin 1.0 l/ha	-	-
12.	-	-	Azoxystrobin 1.0 l/ha	-
13.	-	-	-	Azoxystrobin 1.0 l/ha
14.	-	-	-	-
15.	-	-	Cyprodinil 0.5 kg/ha	Azoxystrobin 0.5 l/ha

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

Active ingredient	*Product*	*Manufacturer*	*g a.i./ha*
Prochloraz	Sportak 45	AgrEvo	405
Azoxystrobin	Amistar	Zeneca	250
Cyprodinil	Unix	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).

Table 2.
SAMPLING SUMMARY

Assessment date	Treatments for visual assessment	Treatments for PCR assessment
Assessment 1	1, 14	1,14
Assessment 2	1, 2, 6, 8,10, 14	1, 14
Assessment 3	1, 2, 4, 5, 6, 7, 8, 10, 11, 14	1, 2, 4, 5, 6, 7, 8, 10, 11, 14
Assessment 4	1, 2, 4, 5, 6, 7, 8, 10, 11, 12, 14	1, 14
Assessment 5	1 to 14	1 to 14
Assessment 6	1 to 14	1, 14
Assessment 7	1 to 14	1 to 14

Table 3.
Assessments dates and growth stages 1998

Assessment	Sampling date	Growth stage
Assessment 1	17 Feb 98	21/22
Assessment 2	15 Apr 98	30
Assessment 3	06 May 98	31
Assessment 4	20 May 98	33/37
Assessment 5	23 Jun 98	59
Assessment 6	20 Jul 98	71/73
Assessment 7	28 Aug 98	90

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

PCR diagnostics were used to study the progress of the eyespot and sharp eyespot epidemics, 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* and *Rhizoctonia solani.* A competitive PCR technique was used at the John Innes Centre, Norwich 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 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.

5. RESULTS

Table 4.
Visual eyespot
% Incidence GS 22-37, % Index GS 59 - 90

Treatment	Treatment / growth stage	Growth stage of assessment 21/22	30	31	33/37	59	71/73	90
T1	UT	5.25	9.75	9.50	7.75	22.3	51.7	53.7
T2	P/25	*	7.25	8.00	8.75	14.0	35.7	47.0
T3	C/32	*	*	*	*	5.3	26.7	53.3
T4	P/30 + C/32	*	*	10.2	5.75	6.7	32.7	50.0
T5	C/30 + P/32	*	*	7.50	5.00	11.7	42.0	50.0
T6	P/25 + P/31	*	9.67	9.25	9.75	16.3	35.7	51.3
T7	C/30 + C/32	*	*	6.50	3.75	15.0	32.0	59.0
T8	P +A/25	*	2.75	8.25	8.75	22.7	48.0	55.3
T9	C+A/32	*	*	*	*	7.0	38.7	59.3
T10	A/25	*	4.00	5.75	5.75	22.0	50.3	55.0
T11	A/30	*	*	6.25	3.00	23.0	51.0	57.7
T12	A/31	*	*	*	3.50	20.3	47.3	53.0
T13	A/32	*	*	*	*	19.0	50.7	55.7
T14	UT	6.50	6.75	9.00	8.25	30.0	42.7	50.3
T15	C/31+A/32	*	*	*	3.00	13.7	50.0	55.7
SED		0.990	1.696	2.683	1.241	6.20	10.72	13.69
P		0.253	0.004	0.757	<0.001	0.008	0.314	1.000

Code	*Active ingredient*	*Product*
P	Prochloraz	Sportak 45
A	Azoxystrobin	Amistar
C	Cyprodinil	Unix

Single products applied at full rate, tank mix or split application products applied at half rate each

Eyespot was assessed visually to be present in the trial at very low levels until flag leaf emergence (GS 33 - 37) and did not exceed an incidence of 10% until the heads were fully emerged in the crop (GS 59). By the end of the season the index was over 50% in nearly all treated and untreated plots which represented a serious eyespot epidemic. The cyprodinil treatments applied at GS 32 (treatment numbers T3 and T9) gave the largest significant reduction in eyespot levels at GS 59. This reduction was still apparent visually at GS 71/73 when full rate cyprodinil (T3) gave the largest reduction in eyespot compared to the untreated plots. The treatments with half rate cyprodinil applied (T7 and T9) were not as good at this timing as the full rate cyprodinil. All the prochloraz and cyprodinil treatments gave some reduction in eyespot compared to the untreated. At GS 90 stems were dying off and eyespot lesions at this time were very advanced and usually included symptoms of Fusarium so differences between treatments were not visually apparent

Table 5.
Visual Sharp eyespot
% Incidence GS 22-37, % Index GS 59 - 90

Treatment	Treatment / growth stage	Growth stage of assessment 21/22	30	31	33/37	59	71/73	90
T1	UT	8.25	0	1.25	1.00	1.67	1.00	0.33
T2	P/25	*	0	0.50	0.25	0.67	1.00	0.67
T3	C/32	*	*	*	*	2.67	2.33	1.00
T4	P/30 + C/32	*	*	0.50	0.50	1.00	0.33	2.00
T5	C/30 + P/32	*	*	0.25	0.25	3.33	4.00	2.33
T6	P/25 + P/31	*	0	0.50	0.25	1.00	2.67	1.67
T7	C/30 + C/32	*	*	0.25	0.00	1.67	4.00	5.33
T8	P +A/25	*	0	0.00	2.75	3.00	3.33	1.00
T9	C+A/32	*	*	*	*	0.67	0.33	0.33
T10	A/25	*	0	0.00	0.00	1.33	4.00	1.00
T11	A/30	*	*	0.75	0.00	0.33	1.33	0.67
T12	A/31	*	*	*	0.00	1.33	1.33	1.33
T13	A/32	*	*	*	*	1.00	0.67	0.00
T14	UT	5.25	0	0.50	0.25	3.00	2.00	0.33
T15	C/31+A/32	*	*	*	3.00	0.67	1.00	0.00
SED		7.246	-	0.428	1.619	1.189	1.636	1.168
P		0.693	-	0.199	0.586	0.213	0.211	0.007

Code	*Active ingredient*	*Product*
P	Prochloraz	Sportak 45
A	Azoxystrobin	Amistar
C	Cyprodinil	Unix

Single products applied at full rate , tank mixed or split application products applied at half rate each.

Sharp eyespot was assessed visually to be present at extremely low levels throughout the season. Initial lesions present at tillering were shed with the lower leaves and after this timing levels only just exceeded 5% in the worst affected plots. Differences between treatments were never significant but tended to be higher in those treatments that had shown common eyespot control (T3 to T9).

Table 6.
Visual Fusarium
% Incidence GS 22-37, % Index GS 59 - 90

Treatment	Treatment / growth stage	Growth stage of assessment 21/22	30	31	33/37	59	71/73	90
T1	UT	12.0	11.5	16.8	16.0	30.0	58.3	51.3
T2	P/25	*	15.5	17.2	18.2	32.3	59.3	41.3
T3	C/32	*	*	*	*	26.0	48.3	44.7
T4	P/30 + C/32	*	*	15.5	11.8	41.3	54.0	44.7
T5	C/30 + P/32	*	*	14.7	9.75	24.7	50.3	49.0
T6	P/25 + P/31	*	15.0	17.2	14.8	33.7	59.0	49.0
T7	C/30 + C/32	*	*	17.5	9.25	21.7	46.0	49.7
T8	P +A/25	*	11.8	14.2	17.0	30.0	58.3	51.3
T9	C+A/32	*	*	*	*	26.0	53.3	50.0
T10	A/25	*	12.0	12.0	13.2	32.0	62.3	51.3
T11	A/30	*	*	16.2	8.00	29.0	54.7	48.0
T12	A/31	*	*	*	9.00	27.7	57.0	51.3
T13	A/32	*	*	*	*	24.0	46.7	45.7
T14	UT	14.8	15.0	15.8	14.5	29.0	57.0	51.7
T15	C/31+A/32	*	*	*	12.0	26.7	51.0	41.0
SED		5.089	0.439	1.989	1.963	7.71	5.56	8.54
P		0.608	0.395	0.204	<0.001	0.696	0.111	0.977

Code	*Active ingredient*	*Product*
P	Prochloraz	Sportak 45
A	Azoxystrobin	Amistar
C	Cyprodinil	Unix

Single products applied at full rate , tank mixed or split application products applied at half rate each.

Fusarium levels in the plots increased steadily throughout the season until watery ripe (GS 71 - 73). At the GS 33/37 assessment the split cyprodinil treatments and the azoxystrobin treatments at GS 30 and 31 (T5, T7, T11 and T12) gave a significant reduction in Fusarium levels. Differences later in the season were not significant but the lowest levels of Fusarium at GS 71/73 were found in the full rate cyprodinil treatment (T3), in the split cyprodinil treatment (T7) as well as in the latest azoxystrobin treatment (T13).

Table 7.
Lodging and yield

Treatment	Treatment / growth stage	Growth stage of assessment Lodging %	Yield t/ha
T1	UT	48.8	3.67
T2	P/25	75.0	3.75
T3	C/32	48.8	4.59
T4	P/30 + C/32	31.8	4.73
T5	C/30 + P/32	62.5	4.39
T6	P/25 + P/31	63.8	3.93
T7	C/30 + C/32	42.5	4.57
T8	P +A/25	60.0	3.74
T9	C+A/32	22.0	5.42
T10	A/25	69.5	3.82
T11	A/30	28.8	4.98
T12	A/31	28.8	4.89
T13	A/32	25.0	5.21
T14	UT	63.8	3.76
T15	C/31+A/32	27.5	5.23
SED		12.31	0.172
P		<0.001	<0.001

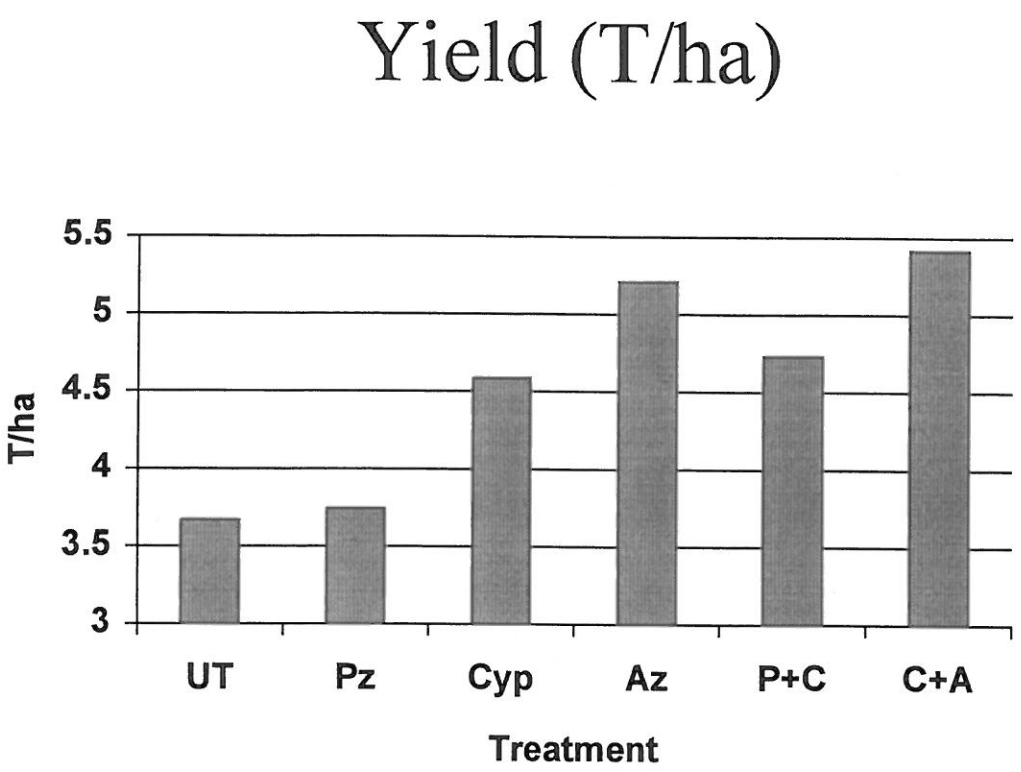
Code	*Active ingredient*	*Product*
P	Prochloraz	Sportak 45
A	Azoxystrobin	Amistar
C	Cyprodinil	Unix

Single products applied at full rate, tank mixed or split application products applied at half rate each.

There were high levels of root lodging in the trial at the end of the season. All the azoxystrobin treatments significantly reduced this, with the later applied treatments showing the largest reduction in lodging.

Yields were low as plots were not over sprayed to control foliar disease. Azoxystrobin showed the largest yield increase over the untreated and this increase was greatest at the GS 32 application (T13). The cyprodinil treatments T3, T4 and T7 also gave yield increases of around a tonne (Figure 1).

Figure 1



The highest yielding treatment was the cyprodinil and azoxystrobin tank mix applied at GS 32. Cyprodinil at GS 32 and prochloraz followed by cyprodinil also increased yield.

Table 10.
W strain PCR analysis
(Fungal DNA (ng per mg plant dry weight)

Treatment	Treatment / growth stage	Growth stage of assessment 21/22	30	31	33/37	59	71/73	90
T1	UT	0	0	0	0	0	0.0012	0.0008
T2	P/25	0	0	0	0	0	*	0.0004
T3	C/32	0	0	0	0	0	*	0.0008
T4	P/30 + C/32	0	0	0	0	0	*	0.0001
T5	C/30 + P/32	0	0	0	0	0	*	0.0008
T6	P/25 + P/31	0	0	0	0	0	*	0.0004
T7	C/30 + C/32	0	0	0	0	5.0×10^{-5}	*	0.0015
T8	P +A/25	0	0	0	0	1.6×10^{-4}	*	0.0010
T9	C+A/32	0	0	0	0	5.0×10^{-5}	*	0.0015
T10	A/25	0	0	0	0	8.2×10^{-5}	*	0.0055
T11	A/30	0	0	0	0	5.0×10^{-5}	*	0.0084
T12	A/31	0	0	0	0	5.0×10^{-5}	*	0.0080
T13	A/32	0	0	0	0	5.5×10^{-4}	*	0.0251
T14	UT	0	0	0	0	4.9×10^{-4}	0.0031	0.0055
SED						-	0.00244	0.00514
P						-	0.470	0.001

Code	*Active ingredient*	*Product*
P	Prochloraz	Sportak 45
A	Azoxystrobin	Amistar
C	Cyprodinil	Unix

Single products applied at full rate , tank mixed or split application products applied at half rate each

Levels of W strain eyespot were very low in the trial. No W strain was detected until GS 59 and levels remained very low until the end of the season. Levels of DNA measured were very variable and one treatment was analysed as having higher levels at the end of the season which gave an apparently higher value for T13.

Table 9.
R strain PCR analysis
(Fungal DNA (ng per mg plant dry weight)

		Growth stage of assessment						
Treat ment	Treatment / growth stage	21/22	30	31	33/37	59	71/73	90
T1	UT	0	0	0	0	0	0.0722	0.1919
T2	P/25	0	0	0	0	0	*	0.0863
T3	C/32	0	0	0	0	0	*	0.1264
T4	P/30 + C/32	0	0	0	0	0	*	0.0480
T5	C/30 + P/32	0	0	0	0	0	*	0.1851
T6	P/25 + P/31	0	0	0	0	0	*	0.1637
T7	C/30 + C/32	0	0	0	0	0.0003	*	0.0715
T8	P +A/25	0	0	0	0	0.0033	*	0.0592
T9	C+A/32	0	0	0	0	0.0030	*	0.0406
T10	A/25	0	0	0	0	0.0091	*	0.1213
T11	A/30	0	0	0	0	0.0036	*	1.0589
T12	A/31	0	0	0	0	0.0051	*	0.1766
T13	A/32	0	0	0	0	0.0002	*	0.2009
T14	UT	0	0	0	0	0.0001	0.050	0.1262
SED						0.00202	0.0500	0.222
P						0.001	0.678	0.008

Code	*Active ingredient*	*Product*
P	Prochloraz	Sportak 45
A	Azoxystrobin	Amistar
C	Cyprodinil	Unix

Single products applied at full rate , tank mixed or split application products applied at half rate each.

R strain eyespot was not detected in the plots at measurable levels until GS 59. Levels between plots at this time were very variable with the untreated plots showing lower levels of R strain eyespot than many of the treated plots. At the end of the season T11 was the only treatments with significantly higher levels than the other plots. Differences between other treatments were not significant but the split cyprodinil treatments tended to have lower levels than the other treatments.