

4.0 MATERIALS AND METHODS

Complete protocols are presented in the Annual Report appendices. The following sections summarise the experimental sites, seasons, treatments, assessments and statistical analysis.

4.1 Sites, years and experiment numbers

Sites and varieties were selected to target specific diseases and the experiment was conducted for three harvest years for *S. nodorum* and two years for brown rust and powdery mildew (Table 4.1).

Table 4.1 Sites, harvest years, experiment numbers and target diseases

Experiment number	Site	Target disease	Harvest year
1	ADAS Rosemaund	Powdery mildew	1997*
2	ADAS Rosemaund	Powdery mildew	1997
3	Morley Research Centre	Brown rust	1997
4	Morley Research Centre	Brown rust	1998
5	ADAS Starcross	<i>S. nodorum</i>	1996
6	ADAS Starcross	<i>S. nodorum</i>	1997
7	ADAS Starcross	<i>S. nodorum</i>	1998
8	ADAS Arthur Rickwood	Powdery mildew	1998

* Experiment funded by MAFF with restricted treatment list.

4.2 Site selection and drilling

Sites were selected according to Standard Operating Procedure (SOP) guidelines following at least a one year non-cereal break and soils were sampled pre-drilling for pH and nutrient status. Plots were drilled at a seed rate calculated from thousand grain weight and according to ADAS guidelines for the soil type and locality. Plot sizes were no smaller than 2m wide x 18m long and were drilled using an Øyjord plot drill or equivalent.

4.3 Experiment Design

Randomised complete block factorial design with three replicates. Where possible, guard plots of a disease resistant variety were drilled alternating with the treated plots.

4.4 Varieties

Varieties were selected for susceptibility to the target disease and resistance to non-target diseases:

Buster at powdery mildew and brown rust target sites.

Brigadier at *S. nodorum* target sites.

4.5 Treatment products, doses, timing and application

Table 4.2 Treatment products, doses and numbers for experiment 2

Treatment Number	Product & dose rate
1.	Alto 100SL 0.8 litre c.p./ha
2.	Alto 100SL 0.6 litre c.p./ha
3.	Alto 100SL 0.4 litre c.p./ha
4.	Alto 100SL 0.2 litre c.p./ha
5.	Opus: 1.0 litre c.p./ha
6.	Opus: 0.75 litre c.p./ha
7.	Opus: 0.5 litre c.p./ha
8.	Opus: 0.25 litre c.p./ha
9.	Unix (cyprodinil): 1.0 kg c.p./ha
10.	Unix (cyprodinil): 0.75 kg c.p./ha
11.	Unix (cyprodinil): 0.5 kg c.p./ha
12.	Unix (cyprodinil): 0.25 kg c.p./ha
13.	Corbel 1.0 litre c.p./ha
14.	Corbel 0.75 litre c.p./ha
15.	Corbel 0.5 litre c.p./ha
16.	Corbel 0.25 litre c.p./ha
17.	Fortress (quinoxifen) 0.3 litre c.p./ha
18.	Fortress (quinoxifen) 0.225 litre c.p./ha
19.	Fortress (quinoxifen) 0.15 litre c.p./ha
20.	Fortress (quinoxifen) 0.075 litre c.p./ha
21.	Patrol 1.0 litre c.p./ha
22.	Patrol 0.75 litre c.p./ha
23.	Patrol 0.5 litre c.p./ha
24.	Patrol 0.25 litre c.p./ha
25.	Ensign (kresoxim-methyl + fenpropimorph) 1.0 litre c.p./ha
26.	Ensign (kresoxim-methyl + fenpropimorph) 0.75 litre c.p./ha
27.	Ensign (kresoxim-methyl + fenpropimorph) 0.5 litre c.p./ha
28.	Ensign (kresoxim-methyl + fenpropimorph) 0.25 litre c.p./ha
29.	Opus Team 1.5 litres c.p./ha
30.	Opus Team 1.125 litre c.p./ha
31.	Opus Team 0.75 litre c.p./ha
32.	Opus Team 0.375 litre c.p./ha
33.	Amistar (azoxystrobin) + Corbel 1.0 + 0.5 litres c.p./ha
34.	Amistar (azoxystrobin) + Corbel 0.75 + 0.375 litres c.p./ha
35.	Amistar (azoxystrobin) + Corbel 0.5 + 0.25 litres c.p./ha
36.	Amistar (azoxystrobin) + Corbel 0.25 + 0.125 litres c.p./ha
37.	Untreated
38.	Untreated

Table 4.3 Treatment products, doses and numbers for experiment 3

Treatment Number	Product & dose rate
1.	Alto 100SL 0.8 litre c.p./ha
2.	Alto 100SL 0.6 litre c.p./ha
3.	Alto 100SL 0.4 litre c.p./ha
4.	Alto 100SL 0.2 litre c.p./ha
5.	Opus: 1.0 litre c.p./ha
6.	Opus: 0.75 litre c.p./ha
7.	Opus: 0.5 litre c.p./ha
8.	Opus: 0.25 litre c.p./ha
9	Corbel 1.0 litre c.p./ha
10	Corbel 0.75 litre c.p./ha
11	Corbel 0.5 litre c.p./ha
12	Corbel 0.25 litre c.p./ha
13	Folicur 1.0 litre c.p./ha
14	Folicur 0.75 litre c.p./ha
15	Folicur 0.5 litre c.p./ha
16	Folicur 0.25 litre c.p./ha
17	Patrol 1.0 litre c.p./ha
18	Patrol 0.75 litre c.p./ha
19	Patrol 0.5 litre c.p./ha
20	Patrol 0.25 litre c.p./ha
21	Amistar (azoxystrobin) 1.0 litre c.p./ha
22	Amistar (azoxystrobin) 0.75 litre c.p./ha
23	Amistar (azoxystrobin) 0.5 litre c.p./ha
24	Amistar (azoxystrobin) 0.25 litre c.p./ha
25	Sanction 0.4 litre c.p./ha
26	Sanction 0.3 litre c.p./ha
27	Sanction 0.2 litre c.p./ha
28	Sanction 0.1 litre c.p./ha
29	Tilt 0.5 litre c.p./ha
30	Tilt 0.375 litre c.p./ha
31	Tilt 0.25 litre c.p./ha
32	Tilt 0.125 litre c.p./ha
33	Untreated
34	Untreated

Table 4.4 Treatment products, doses and numbers for experiment 4

Treatment Number	Product & dose rate
1.	Alto 100SL 0.8 litre c.p./ha
2.	Alto 100SL 0.6 litre c.p./ha
3.	Alto 100SL 0.4 litre c.p./ha
4.	Alto 100SL 0.2 litre c.p./ha
5.	Opus: 1.0 litre c.p./ha
6.	Opus: 0.75 litre c.p./ha
7.	Opus: 0.5 litre c.p./ha
8.	Opus: 0.25 litre c.p./ha
9	Corbel 1.0 litre c.p./ha
10	Corbel 0.75 litre c.p./ha
11	Corbel 0.5 litre c.p./ha
12	Corbel 0.25 litre c.p./ha
13	Folicur 1.0 litre c.p./ha
14	Folicur 0.75 litre c.p./ha
15	Folicur 0.5 litre c.p./ha
16	Folicur 0.25 litre c.p./ha
17	Neon (spiromaxamine) 1.5 litre c.p./ha
18	Neon (spiromaxamine) 1.125 litre c.p./ha
19	Neon (spiromaxamine) 0.75 litre c.p./ha
20	Neon (spiromaxamine) 0.375 litre c.p./ha
21	Amistar (azoxystrobin) 1.0 litre c.p./ha
22	Amistar (azoxystrobin) 0.75 litre c.p./ha
23	Amistar (azoxystrobin) 0.5 litre c.p./ha
24	Amistar (azoxystrobin) 0.25 litre c.p./ha
25	Caramba (metconazole) 1.5 litre c.p./ha
26	Caramba (metconazole) 1.125 litre c.p./ha
27	Caramba (metconazole) 0.75 litre c.p./ha
28	Caramba (metconazole) 0.375 litre c.p./ha
29	Landmark 1.0 litre c.p./ha
30	Landmark 0.75 litre c.p./ha
31	Landmark 0.5 litre c.p./ha
32	Landmark 0.25 litre c.p./ha
33	Corbel 2.0 litre c.p./ha
34	Amistar 2.0 litre c.p./ha
35	Landmark 2.0 litre c.p./ha
36	Alto 1.6 litre c.p./ha
37	Untreated
38	Untreated

Table 4.5 Treatment products, doses and numbers for experiment 5

Treatment Code	Fungicide (active ingredient)	Commercial Product (c.p.)	Dose (c.p. /ha)
1	untreated	-	-
2	untreated	-	-
3	untreated	-	-
4	chlorothalonil	Bravo 500	0.5 litre
5	chlorothalonil	Bravo 500	1.0 litre
6	chlorothalonil	Bravo 500	1.5 litre
7	chlorothalonil	Bravo 500	2.0 litre
8	cyproconazole	Alto100SL	0.2 litre
9	cyproconazole	Alto100SL	0.4 litre
10	cyproconazole	Alto100SL	0.6 litre
11	cyproconazole	Alto100SL	0.8 litre
12	cyprodinil	Unix	0.25 kg
13	cyprodinil	Unix	0.5 kg
14	cyprodinil	Unix	0.75 kg
15	cyprodinil	Unix	1.0 kg
16	epoxiconazole	Opus	0.25 litre
17	epoxiconazole	Opus	0.5 litre
18	epoxiconazole	Opus	0.75 litre
19	epoxiconazole	Opus	1.0 litre
20	flusilazole	Sanction	0.1 litre
21	flusilazole	Sanction	0.2 litre
22	flusilazole	Sanction	0.3 litre
23	flusilazole	Sanction	0.4 litre
24	flutriafol	Pointer	0.25 litre
25	flutriafol	Pointer	0.5 litre
26	flutriafol	Pointer	0.75 litre
27	flutriafol	Pointer	1.0 litre
28	prochloraz	Sportak 45	0.225 litre
29	prochloraz	Sportak 45	0.45 litre
30	prochloraz	Sportak 45	0.675 litre
31	prochloraz	Sportak 45	0.9 litre
32	propiconazole	Tilt 250 EC	0.125 litre
33	propiconazole	Tilt 250 EC	0.25 litre
34	propiconazole	Tilt 250 EC	0.375 litre
35	propiconazole	Tilt 250 EC	0.5 litre
36	tebuconazole	Folicur	0.25 litre
37	tebuconazole	Folicur	0.5 litre
38	tebuconazole	Folicur	0.75 litre
39	tebuconazole	Folicur	1.0 litre

Table 4.6 Treatment products, doses and numbers for experiments 6 and 7

Treatment Code	Fungicide (active ingredient)	Commercial Product (c.p.)	Dose (c.p. /ha)
1	untreated	-	-
2	untreated	-	-
3	untreated	-	-
4	chlorothalonil	Bravo 500	0.5 litre
5	chlorothalonil	Bravo 500	1.0 litre
6	chlorothalonil	Bravo 500	1.5 litre
7	chlorothalonil	Bravo 500	2.0 litre
8	kresoxim-methyl + epoxiconazole	Landmark	0.25 litre
9	kresoxim-methyl + epoxiconazole	Landmark	0.5 litre
10	kresoxim-methyl + epoxiconazole	Landmark	0.75 litre
11	kresoxim-methyl + epoxiconazole	Landmark	1.0 litre
12	cyprodinil	Unix	0.25 kg
13	cyprodinil	Unix	0.5 kg
14	cyprodinil	Unix	0.75 kg
15	cyprodinil	Unix	1.0 kg
16	epoxiconazole	Opus	0.25 litre
17	epoxiconazole	Opus	0.5 litre
18	epoxiconazole	Opus	0.75 litre
19	epoxiconazole	Opus	1.0 litre
20	flusilazole	Sanction	0.1 litre
21	flusilazole	Sanction	0.2 litre
22	flusilazole	Sanction	0.3 litre
23	flusilazole	Sanction	0.4 litre
24	kresoxim-methyl + fenpropimorph	Ensign	0.175 litre
25	kresoxim-methyl + fenpropimorph	Ensign	0.35 litre
26	kresoxim-methyl + fenpropimorph	Ensign	0.525 litre
27	kresoxim-methyl + fenpropimorph	Ensign	0.7 litre
28	azoxystrobin	Amistar	0.25 litre
29	azoxystrobin	Amistar	0.5 litre
30	azoxystrobin	Amistar	0.75 litre
31	azoxystrobin	Amistar	1.0 litre
32	metconazole	Caramba	0.375 litre
33	metconazole	Caramba	0.75 litre
34	metconazole	Caramba	1.125 litre
35	metconazole	Caramba	1.5 litre
36	tebuconazole	Folicur	0.25 litre
37	tebuconazole	Folicur	0.5 litre
38	tebuconazole	Folicur	0.75 litre
39	tebuconazole	Folicur	1.0 litre

Table 4.7 Treatment products, doses and numbers for experiment 8

Treatment number	Product & dose rate
1.	Alto 100SL 0.8 litre c.p./ha
2.	Alto 100SL 0.6 litre c.p./ha
3.	Alto 100SL 0.4 litre c.p./ha
4.	Alto 100SL 0.2 litre c.p./ha
5.	Opus: 1.0 litre c.p./ha
6.	Opus: 0.75 litre c.p./ha
7.	Opus: 0.5 litre c.p./ha
8.	Opus: 0.25 litre c.p./ha
9.	Unix (cyprodinil): 1.0 kg c.p./ha
10.	Unix (cyprodinil): 0.75 kg c.p./ha
11.	Unix (cyprodinil): 0.5 kg c.p./ha
12.	Unix (cyprodinil): 0.25 kg c.p./ha
13.	Neon (spiroxamine) 1.5 litre c.p./ha
14.	Neon (spiroxamine) 1.125 litre c.p./ha
15.	Neon (spiroxamine) 0.75 litre c.p./ha
16.	Neon (spiroxamine) 0.375 litre c.p./ha
17.	Fortress (quinoxifen) 0.3 litre c.p./ha
18.	Fortress (quinoxifen) 0.225 litre c.p./ha
19.	Fortress (quinoxifen) 0.15 litre c.p./ha
20.	Fortress (quinoxifen) 0.075 litre c.p./ha
21.	Patrol 1.0 litre c.p./ha
22.	Patrol 0.75 litre c.p./ha
23.	Patrol 0.5 litre c.p./ha
24.	Patrol 0.25 litre c.p./ha
25.	Ensign (kresoxim-methyl + fenpropimorph) 0.7 litre c.p./ha
26.	Ensign (kresoxim-methyl + fenpropimorph) 0.525 litre c.p./ha
27.	Ensign (kresoxim-methyl + fenpropimorph) 0.35 litre c.p./ha
28.	Ensign (kresoxim-methyl + fenpropimorph) 0.175 litre c.p./ha
29.	Opus Team 1.5 litres c.p./ha
30.	Opus Team 1.125 litre c.p./ha
31.	Opus Team 0.75 litre c.p./ha
32.	Opus Team 0.375 litre c.p./ha
33.	Amistar (azoxystrobin) + Corbel 1.0 + 0.5 litres c.p./ha
34.	Amistar (azoxystrobin) + Corbel 0.75 + 0.375 litres c.p./ha
35.	Amistar (azoxystrobin) + Corbel 0.5 + 0.25 litres c.p./ha
36.	Amistar (azoxystrobin) + Corbel 0.25 + 0.125 litres c.p./ha
37.	Untreated
38.	Untreated

Fungicides were applied at GS 37, when the ligule of leaf 2 was visible on the majority of main tillers, at brown rust sites, and GS33 at powdery mildew sites. The *S. nodorum* target experiments received a single spray at GS33 in 1996, GS39 in 1997 and two-spray programme at GS 32 and GS43-57 in 1998. Treatments were applied using a hand-held pressurised sprayer of the OPS/MDM type and were applied in 200-250 litres of water per hectare, using low drift nozzles selected to produce a medium spray quality at 200-300 kPa pressure.

Other treatments (fertiliser, trace elements, herbicides, insecticides, growth regulators, molluscicides) followed standard farm practice.

4.6 Assessments and records

4.6.1 Agronomic details

Site, soil and crop details were recorded.

4.6.2 Meteorological data

Meteorological data from crop emergence to harvest were recorded using in-crop Delta-T data loggers or at on-site Meteorological Office stations.

4.6.3 Assessment of leaf diseases and green leaf area (GLA)

Pre-treatment disease and GLA assessments were made immediately prior to treatment. 50 main tillers were randomly sampled across the whole of the 'test' variety plot area and the assessments described below recorded (on all leaf layers with an average of >25% GLA remaining).

Disease severity and % GLA were recorded on all green leaves on 10 main tillers per plot on two occasions after treatment. The precise timing of these assessments was adjusted to optimise recording of treatment differences. The first assessment aimed to record treatment differences on leaves 3 and 4, before senescence and at the same time differences were becoming established on the upper leaves. The second assessment aimed at recording treatment effects on leaves 1 & 2.

Disease severity was defined as the percentage leaf area affected by disease, including chlorotic and necrotic areas attributable to disease;

4.6.4 Ear diseases

Diseases were assessed on 10 ears per plot at GS 85, if more than 5% ear area or more than five grain sites per ear were affected in the untreated controls.

4.6.5 Stem bases diseases

Stem-base diseases were assessed on 25 tillers from the trial area at GS 31.

At GS 75, stem-base diseases were assessed in all plots on 25 tillers per plot, if in untreated plots, >25% tillers were affected by moderate or severe lesions of any disease or if >10% tillers were affected by severe lesions of any disease.

4.6.6 Harvest

Whole plots were harvested. Grain yield was adjusted to 85% dry matter. Grain specific weight and thousand grain weight were adjusted to 85% dry matter.

4.7 Standard Operating Procedures List

Work was conducted according to the ADAS Standard Operating Procedures listed in the protocol.

4.8 Data handling

Disease, green leaf area and yield/grain quality measurements were collected either manually or directly on to portable computers and transferred onto MINITAB or EXCEL work files after collection.

4.9 Statistical analysis

Data were analysed using Genstat 5.

4.9.1 Individual Assessments

Each assessment (site, season, variate, date, leaf layer) was analysed by analysis of variance and the validity of the analysis was checked by examination of residuals. Normal plots, histograms and plots of residuals versus fitted values were used to assess the normality assumption and any requirement for transformation.

Outliers were identified from the above plots, and from graphs of residuals versus fungicide and residuals versus dose. A small number of extreme outliers were removed from the data after consultation as to the cause.

In some cases, plots of residuals versus plot number showed a linear trend in the residuals within some of the blocks. These trends were removed by using covariates on plot number within each block.

Variates which did not contribute useful information were excluded from further analysis. These were defined to be variates for which there were no significant treatment effects or interactions, disease variates for which there was less than an average of 5% disease on the untreated plots, and green leaf areas for which there was more than an average of 90% green leaf area on the untreated plots.

For disease variates which did contribute useful information, dose-response curves were plotted for each fungicide, using the treatment means (adjusted for covariates if

appropriate). Exponential curves of the form $y = a + be^{kx}$, where $y = \% \text{ disease}$ and $x = \text{proportion of recommended dose}$, were fitted. The three parameter exponential was the most parsimonious function, able to describe the variation in dose-response seen in the data. All of the parameters have biological meaning. Exponential curves were also fitted to green leaf areas and harvest variates. Since the untreated (dose=0) data point for each fungicide is the mean of the same six plot values, the curves were constrained to pass through this point.

4.9.2 Over-assessment means

For disease variates, assessments were split into those representing either eradicator or protectant activity of the fungicides. All assessments on a leaf layers were ascribed to the eradicator category if the leaf had been emerged sufficiently long for infection to have become established, by the time treatments were applied. Assessments on leaf layers which were treated soon after emergence were ascribed to the protectant category. Exponential curves were fitted to means over all sites, seasons, dates and leaf layers for each fungicide and each type of activity, regardless of the closeness of the fit of the curves to the individual assessments. Inclusion of all the data was considered appropriate, as a lack of fit was often the result of poor disease control, and was thus a true reflection of the performance of the fungicide. The curves were again constrained to pass through the untreated (dose=0) point. Repeat assessments on the same leaf layer within a site/season are likely to be highly correlated. Hence, such assessments were averaged before the overall means were calculated.

Where analysis indicated that there was not interaction between the category of the data (eradicator or protectant) and fungicide, eradicator and protectant data were combined.

In a relatively small number of cases, the curve fitting process failed to converge. In other cases, the fitting process resulted in illogical curves. These are indicated in the figures by 'data not fitted' and 'refer to text', respectively.

Where dose-response curvature was severe, the fitting procedure was unable to provide a reliable estimate of where maximum curvature occurred, except to identify that it lay between doses 0 and 0.25. A limit for k of -20 has been imposed in the figures to reflect these cases in a biologically realistic form.

Green leaf area over-assessment means were calculated from the same site, season, date and leaf layer assessment combinations as the relevant disease means. Various combinations of site and season means were calculated for the harvest variables, for comparison with disease and green leaf area means. Exponential curves were fitted to green leaf area and harvest variates.