8.0 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.

8.1 Sites, years and experiment numbers

Sites and varieties were selected to target specific diseases and the experiment was conducted for two harvest years for powdery mildew and brown rust, and three harvest years for *Septoria* (*Stagonospora*) *nodurum*.

Table 8.1 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	1998
3	Morley Research Centre	Brown rust	1997
4	Morley Research Centre	Brown rust	1998
5	ADAS Starcross	*S. nodorum*	1996
6	ADAS Starcross	*S. nodorum*	1997
7	ADAS Starcross	*S. nodorum*	1998

* Disease failed to develop at this site.

8.2 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.

8.3 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.

8.4 Varieties

The varieties shown in Table 8.2 were selected to provide a range of levels of resistance to the target disease and, as far a possible, minimise interference by non-target diseases.

Table 8.2 Winter wheat varieties selected for each site

	Variety	Expts.1&2	Expt.3	Expt.4	Expts.5,6,7
1	Admiral				✓
2	Brigadier	✓	✓		✓
3	Mercia				✓
4	Hunter	✓	✓	✓	✓
5	Hussar				✓
6	Spark		✓	✓	✓
7	Buster	✓	✓	✓	
8	Beaufort	✓			
9	Genesis	✓			
10	Hereward	✓			
11	Rialto		✓	✓	
12	Abbot			✓	
13	Riband		✓	✓	

8.5 Treatment products and doses

Tebuconazole (as c.p. Folicur, Bayer UK) and fenpropidin (Patrol, Zeneca) were used, for continuity with the first three years of appropriate doses experimentation (HGCA Report No. 166), at the range of doses shown in Table 8.3.

Table 8.3 Fungicide products and doses applied at each site

Trt. No.	Experiments 5,6&7	Experiments 1,2,3&4
1	Untreated	Untreated
2	Folicur 1.0 litre c.p./ha	Folicur 1.0 litre + Patrol 0.7 litre c.p./ha
3	Folicur 0.75 litre c.p./ha	Folicur 0.75 litre + Patrol 0.525 litre c.p./ha
4	Folicur 0.5 litre c.p./ha	Folicur 0.5 litre + Patrol 0.35 litre c.p./ha
5	Folicur 0.25 litre c.p./ha	Folicur 0.25 + Patrol 0.175 litre c.p./ha

Fungicide treatments were applied at GS 37 in experiments 1,2,3 and 4, GS33 in experiment 5, GS39 in experiment 6, and as a two-spray programme at GS 32 and GS 43-57 in experiment 7. Sprays 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 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.

8.6 Assessments and records

8.6.1 Agronomic details

Site, soil and crop details were recorded.

8.6.2 Meteorological data

Meteorological data from crop emergence to harvest were recorded using in-crop Delta-T data loggers or standard Meteorological Office recording sites.

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

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

At approximately 21 days and 42 days after treatment (for experiments 1, 2, 3 & 4) or 28 and 50 days after treatment (experiments 5, 6 & 7) disease severity and percentage green leaf area (GLA) were recorded on all green leaves on 10 main tillers per plot. 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;

8.6.4 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.

8.6.5 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.

8.6.6 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.

8.7 SOP List

Work was conducted according to the ADAS Standard Operating Procedures listed in the protocols (see Annual Report appendices)..

8.8 Data handling

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

8.9 Statistical analysis

Data were analysed using Genstat 5.

8.9.1 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 v 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 variety 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 v 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 variety using the treatment means (adjusted for covariates if appropriate). Exponential curves of the form $y = a+be^{kx}$, where y = % disease and x = proportion of recommended dose were fitted. Examination of the data suggested that a model which allowed the a and b parameters to vary for each variety, but used a common k across all the varieties within an experiment, provided a reasonable description of the data in most cases. This model was used to fit exponential curves to all individual assessments.

Exponential curves were also fitted to green leaf areas and harvest variates.

8.9.2 Over-assessment means

For disease variates, assessments were split into eradicant and protectant categories, as described for the product dose response experiments. Exponential curves were fitted to means over all sites (containing the same varieties), seasons, dates and leaf layers for each variety and each type of activity, regardless of the closeness of the fit of the curves to the individual assessments. 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.

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.

Observation of the fitted response curves did not suggest the presence of variety by eradicant/protectant category interactions for disease or green leaf area variates. The absence of interactions was confirmed by regression analysis on a sub-set of the data; allowing response curve fitting to combined eradicant and protectant data.

In order to identify the way in which the exponential curve parameters were varying across the varieties, an analysis of parallelism was carried out for over-assessment means. Such an analysis fits a sequence of models, of increasing complexity, until allowing extra parameters does not markedly improve the fit of the model. The sequence of models fitted was:

a) common curve for all varieties:

$$y=a+be^{kx}$$

b) separate b parameters (difference between the asymptote and the untreated value) for each variety:

$$y=a+b_ie^{kx}$$

c) separate a (lower asymptote) and b parameters for each variety:

$$y=a_i+b_ie^{kx}$$

d) separate a, b and k (curvature) parameters for each variety:

$$y=a_i+b_ie^{k_i x}$$

Where 'data not fitted' appears in the results section, the model could not be fitted to the data. Where 'refer to text' appears in the results section, Genstat fitted a curve, but the direction of curvature was illogical.