6.0 IMPROVING RESPONSE CURVE PARAMETER ESTIMATION

The precision with which fungicides can be used depends on the precision with which their relative performance can be measured. Here, fungicide performance is described by the parameter estimates a, b and k. Field experiments to gather data from which these parameters can be calculated, are resource intensive - so it is important that parameter estimates are obtained in the most cost effective manner.

6.1 Controlling extraneous variability

During evaluation of efficacy, applications of a given fungicide against a given target disease at a range of sites and seasons, result in a family of different dose-response curves, brought about by the ".....inherent variability, which is characteristic of biological systems..." (Finney, 1993). When comparing the relative performance of fungicides, this variability often results in changes in the rank order of products between experiments. To overcome this variability, estimates of fungicide performance are typically made from trials carried out across many sites and seasons. The work presented by Paveley *et al.* (in press) has shown that the relationship between spray timing and leaf emergence is a major cause of this variation; control of which could reduce the number of experiments required to obtain reliable estimates of efficacy. Most simply, if the leaf layer which has most recently emerged is noted at the time of treatment, then dose-response curves derived from subsequent disease assessments on that leaf layer, should consistently quantify near optimal efficacy. Dose-response curves derived from observations on the leaf layers above and below that layer, quantify protectant and eradicant performance, respectively.

6.2 Minimising dose points

The number of dose treatments required in an experiment depends on the number of parameters to be estimated. Initial observations with conventional triazole fungicides, suggested that the a parameter (which represents the proportion of the pathogen population in an insensitive stage of its life cycle) might be sufficiently stable across products, within an assessment, that it might be considered constant - thus, reducing the number of dose points required to estimate the response-curve. Besides reducing the number of treatment required in future experimentation, this might also allow data collected from other projects, where fewer dose points were measured, to be used. However, the proliferation of novel active ingredients, which vary substantially in their degree of eradicant action, has given rise to substantial variation in a, and therefore b. Similarly, the widening gap between the performance of new and old active ingredients is expressed predominantly through variation in k. Hence, there is now no practicable scope to reduce the number of parameters required to represent fungicide performance - with the possible exception of the estimation of dose response curves for single products across varieties, where constant k values are the rule rather than the exception.

To ensure sufficient degrees of freedom in the analysis, the minimum number of dose treatments is one more than the number of parameters to be estimated. Working at this minimum level is only an option where the there is both some prior knowledge of

the curvature of the dose-response, and reasonable consistency in curvature between fungicides. Neither, pre-requirement is adequately met in the type of dose-response studies reported here, so an additional point is required to account for the range of variation in the dose at which maximum curvature will lie.

A more fruitful line of enquiry is to consider how the dose treatments might be distributed most efficiently, to improve the precision of parameter estimation.

6.3 Optimising the distribution of dose treatments

The optimum distribution of dose treatments is a compromise between need for high dose points to better define the lower asymptote, and low dose points to define curvature. Doses higher than those recommended also carry some risk of crop scorch, prejudicing the accuracy of disease and green leaf area assessments.

To test the risks and benefits of a change in dose treatments, four additional plots per block were included in experiment 4. These plots received a double dose of four of the fungicides in the experiment (Alto, Corbel, Amistar, Landmark), selected to contrast in their k and b parameter estimates, and in the likelihood of crop scorch.

The data were used to test whether the precision and accuracy of parameter estimation would be improved by using an exponential distribution of dose treatments (0, 0.25, 0.5, 1 and 2), rather than a linear distribution (0, 0.25, 0.5, 0.75 and 1).

6.3.1 Statistical method

For each assessment and variate, data were available for each of the four fungicides at six doses (0, 0.25, 0.5, 0.75, 1 and 2). Exponential points, constrained to pass through the untreated point, were fitted to:
i) all six points, giving parameter estimates k_{all} and b_{all}.
ii) data points excluding dose 2, giving parameter estimates k_{exc2} and b_{exc2}.
iii) data points excluding dose 0.75, giving parameter estimates $k_{exc.75}$ and $b_{exc.75}$.

Taking the curve fitted to all six data points to be the best available estimate of the parameters of the exponential curve for each fungicide, we then examined how close the estimates for ii) and iii) were to k_{all} and b_{all}. Closeness was assessed in terms of a mean-square error statistic:

$$MSE = (bias)^2 + variance$$

where bias is estimated by the difference between the estimate from ii) or iii) and the value being estimated (e.g. $k_{exc2} - k_{all}$) and variance is estimated by the square of the standard error (SE) of the estimate from ii) or iii). Thus, MSE incorporates both the accuracy and precision of the estimate.

Data from all the assessments of leaf variates that were included in the over-assessment analysis presented in this report were used.

6.3.2 Results

Differences in MSEs between parameter estimates from linear (excluding dose 2) and exponential (excluding dose 0.75) dose scales are shown in Figures 6.1 and 6.2, for parameters k and b respectively.

Figure 6.1 Scatter diagram of differences in MSEs ($MSEk_{exc2}$ - $MSEk_{exc.75}$) on k.

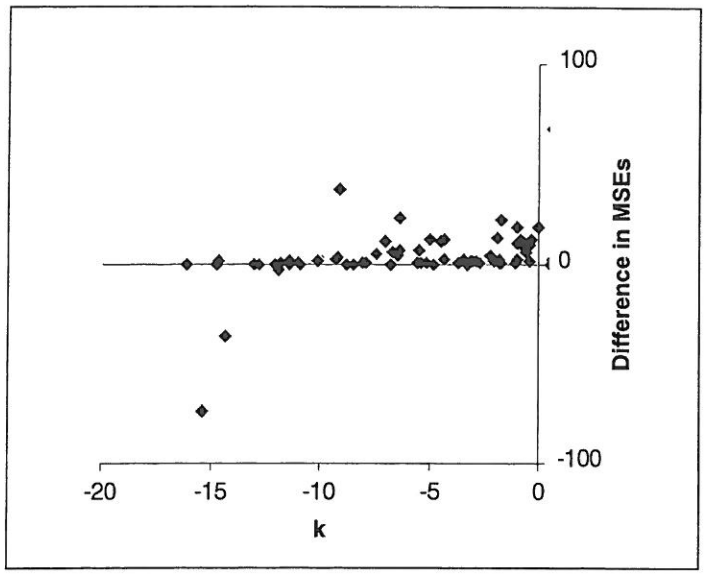
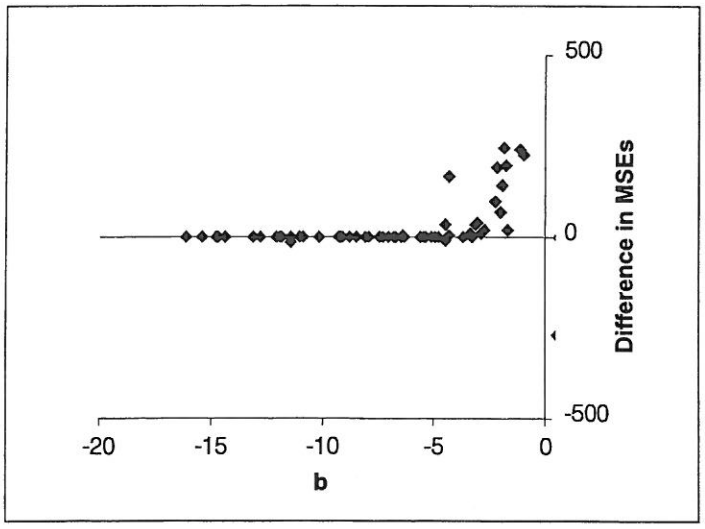


Figure 6.2 Scatter diagram of differences in MSEs ($MSEb_{exc2}$ - $MSEb_{exc.75}$) on b.



For k, differences in MSEs were generally positive, indicating that an exponential distribution of dose treatments (0, 0.25, 0.5, 1 and 2) was usually better than a linear series (0, 0.25, 0.5, 0.75 and 1). This was particularly the case for small negative k values, rather then for large negative k values, i.e. there was less advantage in using the double dose when there was large curvature.

For b, differences in MSEs were generally positive, indicating that an exponential distribution of dose treatments (0, 0.25, 0.5, 1 and 2) was usually better than a linear series (0, 0.25, 0.5, 0.75 and 1). Very large positive values of differences in MSEs (outside the y-axis range in Figure 6.1) were noted in some cases. These were mainly caused by positive estimates of k when dose 2 was excluded.

Field assessments suggested that crop scorch from the double doses was not notably greater than from a single dose and did not adversely affect either the accuracy of assessments or yield.

7.0 RELATING RESPONSES BETWEEN DIFFERENT EXPERIMENTS

To be of enduring value to growers, information on the performance of products needs to be kept current. In particular, novel fungicides will continue to enter the market and their performance relative to products in the existing data set will need to be assessed. It would be inefficient to re-run multi-site experiments with the entire range of existing fungicide active ingredients, each time a new fungicide nears approval, in order to allow a direct comparison. This section describes the development and testing of a technique to allow new fungicides to be assessed in relation to a small number of 'standards', which can then be used to relate the performance of that product to others in the existing data set, or to other novel fungicides assessed in separate projects.

7.1 Statistical method

In a worst case scenario, a number of new fungicides become available over a period of years. In each case, the new fungicide is tested against a small number of 'standards', in randomised and replicated experiments of the standard design described in this report. A table of disease, green area or yield responses to any given dose, for all fungicides and all experimental sites will include empty cells. Comparisons between averages over all experiments will require adjustment to allow for differences in the conditions (particularly disease pressure) experienced at different sites.

The method of fitting constants (FITCON) (Yates, 1933) has been widely used in variety testing for the analysis of incomplete tables of variety by site data (Patterson 1978, 1982). In application to the fungicide problem, if y_{ij} is the response of fungicide I ($1<=i<=m$) in experiment j ($1<=k<=n$), the fungicide means are estimated by fitting the model:

$$y_{ij}=a_i+c_j$$

where a_i is the estimated mean response for fungicide I and c_j the estimated effect of experiment j, by least squares subject to the constraint that the mean response for the standard varieties, that are present in all the experiments, is equal to the unadjusted mean response over all the experiments, i.e. $ai=\Sigma_j\ y_{ij}/n$ for any fungicide that appears in all the experiments.

A representative simulation study was used to examine whether the FITCON method could be applied reliably to the fungicide case.

Data from experiments at ADAS Rosemaund and Morley Research Centre in 1994, 1995 and 1996 (reported in HGCA Report No. 166) were used to test the FITCON method. Yield and percentage *Septoria tritici* data at each of five doses were available for the eight fungicides that were tested in all experiments. *Septoria tritici* was assessed on several leaf layers on several dates throughout the season. Two sets

of fungicide by dose responses were constructed for each experiment: grain yield and percentage septoria tritici for eradicant activity. For each variate, the data were considered as eight fungicides, five doses and six experiments.

For the purposes of the test it was supposed that yield and *Septoria tritici* data were obtained from two projects. In each project, three new fungicides were being considered in each of three experiments. Two standard fungicides, of contrasting mode of action, were also included in each project. This situation was simulated by designating two of the fungicides to be the standards, and three to belong to each project:

Standards: Folicur and Bravo
Project A: Alto, Corbel and Sanction
Project B: Patrol, Pointer and Tilt

After assigning three of the six available experiments to project A and three to project B and deleting the responses for the fungicides not included in a particular project, a table of the responses at a given dose was of the form shown in Table 7.1.

Table 7.1 Table of response data from experiments in two notional projects.

	Experiment					
	Project A			Project B		
Fungicide	1	2	3	4	5	6
Folicur	x	x	x	x	x	x
Bravo	x	x	x	x	x	x
Alto	x	x	x			
Corbel	x	x	x			
Sanction	x	x	x			
Patrol				x	x	x
Pointer				x	x	x
Tilt				x	x	x

The FITCON method was then applied to calculate adjusted fungicide means. This was repeated separately for each dose. Hence, an adjusted dose-response curve for each fungicide was calculated. Exponential curves, constrained to pass through dose=0, were fitted for each fungicide and used to predict the expected response at dose=1.

The ranking of the fungicides using this approach was then compared with that available from applying the same curve fitting procedure to the full dataset (before removal of some responses).

The choice of which experiment to notionally assign to which project could have a large effect on the results. So the calculations were repeated for all 20 ways of dividing the six experiments between the two projects.

7.2 Results

In a perfect world, the rank order of fungicide performance calculated through FITCON would match exactly the rank order calculated from the full data set. Tables 7.2 and 7.3 below show the difference in rank order between the FITCON adjusted performance estimates and estimates from the complete dataset, for each product, for each of the 20 permutations (described above), for eradicant disease control and grain yield, respectively. Zero represents no change in rank order.

Table7.2. Difference in rank order of eradicant control performance from FITCON analysis, compared against estimates from the complete dataset.

	Permutation																			
Product	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Folicur	1	1	0	1	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0
Bravo	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0
Alto	-1	-1	1	-1	-1	0	-1	0	-1	1	-1	0	-1	0	-1	1	0	0	1	1
Corbel	1	0	1	1	1	1	1	0	0	1	0	1	1	-1	-1	0	0	0	1	0
Sanction	0	0	-1	0	0	0	0	0	0	-1	0	0	0	0	0	-1	0	0	-1	-1
Patrol	-2	0	-1	-1	-1	-1	-2	0	0	-1	0	-1	-2	0	0	0	0	0	-1	0
Pointer	0	0	0	0	0	-1	0	-1	0	0	0	-1	0	-1	0	0	-1	0	-1	-1
Tilt	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	1	0	1	1

Table 7.3 Difference in rank order of yield performance from FITCON analysis, compared against estimates from the complete dataset.

	Permutation																			
Product	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Folicur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bravo	0	1	0	1	1	1	1	0	0	0	0	1	0	0	1	0	1	0	1	0
Alto	0	0	-1	0	0	0	0	0	0	0	0	0	0	-1	0	-1	0	0	0	-1
Corbel	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0
Sanction	0	-2	-1	-2	1	1	1	0	-1	0	-1	0	0	-1	-2	-1	1	1	1	0
Patrol	0	0	-1	0	0	0	0	-1	0	0	0	-1	0	-1	0	0	-1	0	0	0
Pointer	0	0	1	0	-1	-1	-1	0	0	0	0	0	0	1	0	1	-1	-1	-1	1
Tilt	0	1	1	1	-1	-1	-1	0	1	0	1	-1	0	1	1	1	-1	0	-1	0

Given that the differences in performance between adjacent products in the rank order were generally small, and that the assessments on which they are based are subject to error variation, the performance of FITCON was good. In 98% of cases the rank position estimated by FITCON was within one position either way of the position estimated from the complete data set.

7.3 Cross-site and season analysis of *Septoria tritici* data

Septoria tritici was present in experiments 1, 3, 4 and 9. In some assessments, the level of other diseases was sufficiently low not to have confounded the dose-response curve. Data from these assessments were therefore added to the *Septoria tritici* data reported in HGCA Report No. 166 and the complete dataset analysed by FITCON

(Tables 7.4 and 7.5). The resulting eradicant and protectant dose-response curves are shown in Figures 7.1 and 7.2, respectively.

Table 7.4 Cross-site parameter estimates for fitted dose response curves - eradicant

Product	Parameter estimates					
	a	b	k	a + b	$a + be^k$	Mean R^2 adjusted
Alto	10.2	21.9	-2.3	32.11	12.5	99.0
Opus	5.4	26.7	-4.6	32.11	5.7	95.0
Bravo	23.0	9.1	-5.7	32.11	23.1	89.5
Corbel	24.6	7.5	-2.0	32.11	25.6	70.8
Folicur	12.1	20.0	-3.7	32.11	12.6	100.0
Patrol	23.8	8.3	-1.4	32.11	25.8	97.2
Pointer	16.9	15.2	-2.0	32.11	19.0	98.8
Sanction	12.8	19.4	-2.3	32.11	14.8	95.2
Tilt	17.1	15.0	-2.7	32.11	18.1	96.6
Bravo+Pointer	16.6	15.5	-6.0	32.11	16.6	-25.1
Bayfidan	24.3	7.8	-2.8	32.11	24.7	-19.3
Unix	32.3	-0.2	4.1	32.11	23.3	73.2
Amistar	19.3	12.8	-4.3	32.11	19.5	68.6
Ensign	12.4	18.7	-3.0	32.11	14.2	81.0
Fortress	32.1	0.0	<-20.0	32.11	*	*
Opus team	8.1	24.0	-6.0	32.11	8.1	60.2
Amistar+Corbel	7.3	24.8	-1.4	32.11	13.5	85.6
Landmark	6.6	25.5	-4.5	32.11	6.8	55.9
Neon	27.6	4.5	-5.2	32.11	27.6	23.4
Caramba	10.9	21.2	-2.6	32.11	12.6	43.6

Table 7.5 Cross site parameter estimates for fitted dose response curves - protectant

Product	Parameter estimates					
	a	b	k	a + b	$a + be^k$	Mean R^2 adjusted
Alto	7.6	17.8	-4.5	25.47	7.8	97.7
Opus	3.4	22.1	-6.9	25.47	3.4	97.6
Bravo	2.1	23.4	-4.0	25.47	2.5	97.8
Corbel	18.1	7.4	-8.8	25.47	18.1	-13.9
Folicur	6.7	18.7	-5.2	25.47	6.9	96.4
Patrol	-62.7	88.2	-0.1	25.47	18.8	37.9
Pointer	13.3	12.1	-5.1	25.47	13.4	66.5
Sanction	9.8	15.7	-3.9	25.47	10.1	47.7
Tilt	8.4	17.1	-3.0	25.47	9.3	69.7
Bravo+Pointer	0.8	24.7	-5.8	25.47	0.8	98.8
Bayfidan	*	*	*	25.47	*	*
Unix	-35.9	61.4	-0.2	25.47	14.5	80.7
Amistar	-2.8	28.2	-1.4	25.47	4.5	91.9
Ensign	6.6	18.8	-2.4	25.47	8.3	95.3
Fortress	-33.7	59.2	-0.2	25.47	17.7	66.0
Opus team	7.2	18.3	-5.8	25.47	7.2	86.4
Amistar+Corbel	3.9	21.5	-2.4	25.47	6.0	78.7
Landmark	4.4	21.1	-8.7	25.47	4.4	79.7
Neon	11.4	14.1	-3.1	25.47	12.2	76.0
Caramba	9.5	16.0	-5.5	25.47	9.6	33.8

Figure 7.1 Protectant dose-response curves for *Septoria tritici* - overall means across 1994 to 1998.

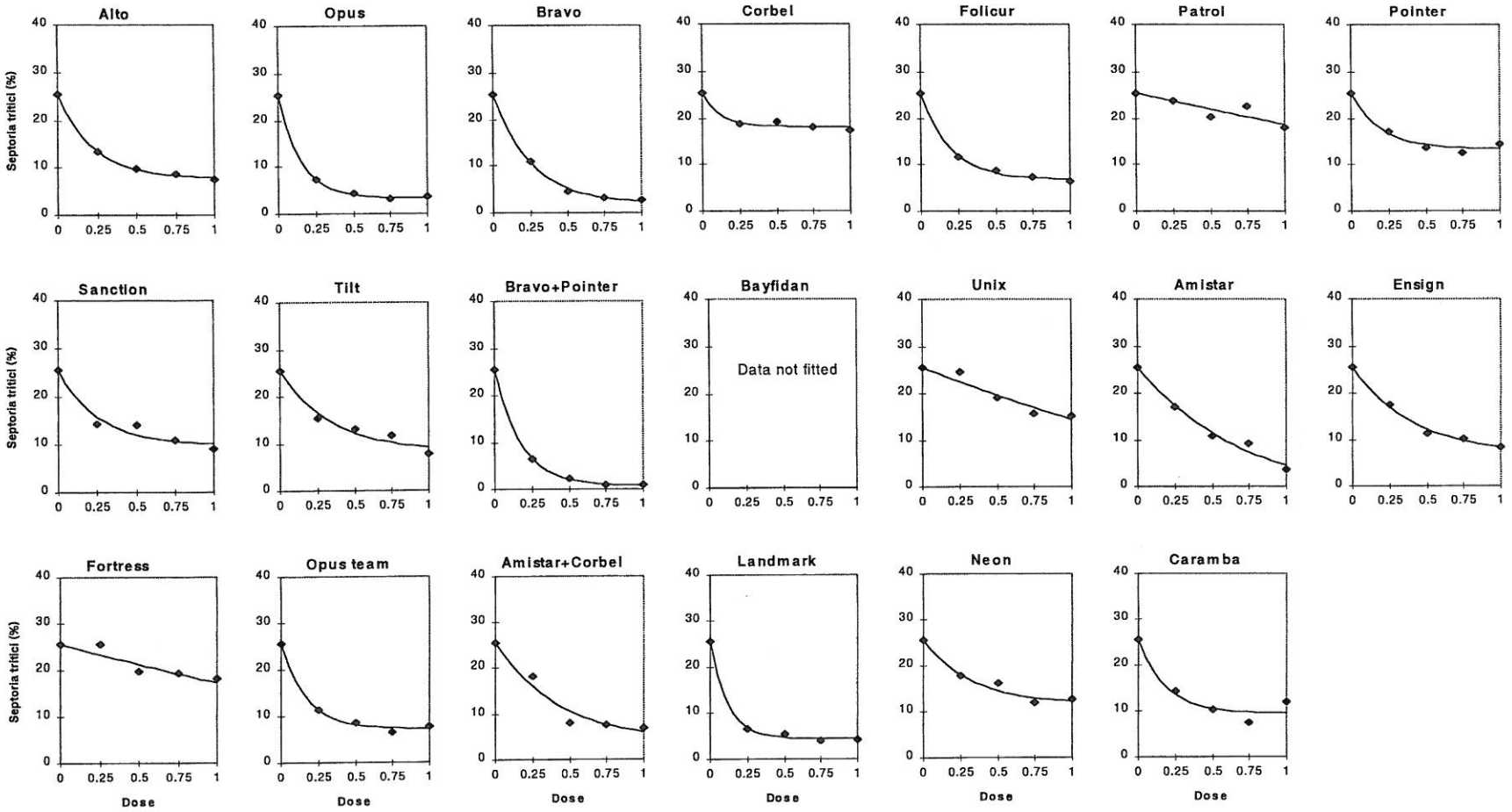


Figure 7.2 Eradicant dose-response curves for *Septoria tritici* - overall means across 1994 to 1998.