

The efficiency with which intercepted radiation was converted to dry matter was the same in all crops, about 1.9 g/MJ. Therefore, the nitrogen fertiliser must have influenced the amount of radiation that was intercepted. In cereals more than 80 per cent of the incident radiation is being intercepted once GAI exceeds about four. There was no difference in GAI until March, when crops that received nitrogen fertiliser had larger GAIs and the differences between the crops was largest at anthesis, when maximum GAI was reached (Table 3). Table 3 also

Table 3 Effect of nitrogen fertiliser on the maximum GAI and the duration of GAI above 4

Crop	Maximum GAI	Duration of GAI>4 (days)
N ₁	5.6	39
N ₂	7.7	70
N ₃	9.1	88

shows that nitrogen fertiliser increased persistence of the GAI, there being a two-fold difference in the duration of GAI above four between the well-fertilised and poorly fertilised crops. As the amount of incident radiation was the same for all crops, the effect of the nitrogen fertiliser was to increase the total amount of radiation intercepted by the crops.

Having identified that the main effect of the nitrogen fertiliser was to increase GAI, is it possible to identify which processes in leaf area production and persistence were affected? The rates at which leaf primordia were initiated on the apex (64 °C days/leaf) and the rate of leaf appearance (104 °C days/leaf) were the same for all three crops and not influenced by nitrogen fertiliser. However, the area of the last four leaves to appear on the mainstem was increased with

Table 4 Effect of nitrogen fertiliser on the size of the last four leaves to appear on the main stem; leaf 14 was the flag leaf

Crop	Area of leaf (cm ² /leaf)			
	11	12	13	14
N ₁	14.6	19.8	27.0	27.8
N ₂	16.1	20.5	22.7	32.3
N ₃	18.2	26.1	31.2	33.3

increasing nitrogen fertiliser (Table 4). Leaf area production was also increased because the number of tillers surviving to produce an ear at anthesis increased from 0.2 tillers/plant for N₁ to 1.1 tillers/plant for N₃.

These results clearly show that the major effect of nitrogen fertiliser on cereal growth and yield is to increase the total amount of radiation intercepted by the crop and not the efficiency with which it is converted to dry matter. This is achieved not by increasing the rate at which leaves emerged nor the number of leaves produced on individual stems, but by increasing the area of individual leaves and the number of ear-bearing stems, via tillering.

Design and interpretation of nitrogen response experiments

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Summary

The investigation of response to nitrogen application may be undertaken at the fundamental, descriptive or predictive level. Fundamental investigation is considered inappropriate for field experiments. The descriptive approach, summarising and discussing results, has been useful in the past but serves only to indicate broad trends. The predictive approach implies the assumption of a model. The parameters of this model are estimated from the data and used to predict future results or to compare experiments. Earlier work in this field is reviewed, and the exponential function modified by the addition of a linear term is proposed as a convenient working model. The properties of this model are discussed and some examples of its use are given. A possible generalisation to a modified logistic function is also suggested. The implications for the conduct of future experiments are considered and it is shown that the rigid approach appropriate for analysis of variance is no longer necessary.

Introduction

In some branches of science and engineering experimental results may be obtained with high precision and repeatability. It is then possible to formulate a law for the process under observation based on the data, and to deduce from this the underlying mechanism. Unfortunately, in agricultural research the results from similar experiments may differ widely, and the formulation of a law is seldom possible. Some of the causes of this variation are obvious, but their effects may be very patchy, even within quite closely adjacent and apparently similar areas. In addition, the response to nitrogen may be highly dependent on other applied treatments or background factors such as previous cropping.

The investigation of crop response to nitrogen application is considered below at three levels, described broadly as fundamental, descriptive and predictive. Models used by previous workers are briefly reviewed, and the modified exponential function is proposed as a useful working model. The merits of this model and the implications for future experiments are then considered.

Fundamental investigation

At the fundamental level the objective is to determine the detailed mechanism of the response. Because of the wide variability of field experiments there is little hope of achieving this objective. Some information may be available from field experiments using the same, or closely adjacent, plots over a long period, but these are still subject to much uncontrolled and local variation. Closely controlled experiments in laboratory conditions will normally be required.

Descriptive analysis

The descriptive level of analysis aims to summarise results, possibly from a wide range of experiments, and to describe empirically the relationship between chosen measures of response and the known features of the experiments. Typical of this approach are the papers by Lessels and Webber (1965) on the effect of nitrogen on spring and winter cereals. These take the yield with zero applied nitrogen as a basis for classifying the results, and discuss the increase in yield resulting from the application of nitrogen at a standard level (usually 0.30 and 0.60 cwt/acre). No model is considered and the quoted values are the average increases in yield over several sites and years. Not surprisingly, the results show many inconsistencies and few clear trends. This may be attributable partly to the rather small number of results involved in most of the averages, and partly to the rather arbitrary division of the data by region, presence or absence of lodging, etc. A further source of difficulty is probably the use of zero applied nitrogen as the reference standard. This is discussed further below.

Analysis of variance

A common adjunct to the descriptive approach is the use of analysis of variance. In its basic form this tests the hypothesis that the applied treatment factors have no effect, by means of the ratios of appropriate mean squares. A significantly large ratio implies that the variation in the observed means of the factor levels is greater than might reasonably be expected to arise by chance. The hypothesis of no real differences is, therefore, untenable and one concludes that real differences do exist. A significant result says nothing about their size or nature, only that on average there are appreciable differences in the effects of the levels of the factor. It may be that yields at all levels differ appreciably from each other, or that yields at most levels are similar but a few differ considerably. The identification of any pattern is a separate matter.

The occurrence of a mean square ratio too small to be considered significant does not imply that the levels of the factor are equal in effect, only that in the conditions of the experiment the observed differences were not unduly large. There may be differences in effects which are masked by the average nature of the test, particularly if most of the levels are similar in effect and only one effect differs appreciably. This one difference may be of considerable interest even though the average difference is relatively small. In addition, if several factors are

involved the main effects are averages over all levels of the other factors. The main effects may then be small even though there are large, but possibly varying, effects at some levels of these other factors. This situation would normally be detected by the occurrence of significantly large interaction terms, provided that the design and analysis permit the separation of such terms.

Very small mean square ratios are an indication of a failure of some part of the model assumptions. The assumed distributional properties may not be valid, the treatment effects may not be additive, or the random components may not be independent, i.e. there may be unsuspected correlations between the observed values of some experimental units. Failures such as this may be evident on inspection of the residuals, particularly when arranged in field order.

Example

As an example of the interpretation of a fairly simple experiment consider the wheat yields of the organic manuring experiment 79/W/RN/12 published in, *Yields of the Field Experiments, 1979*, by Rothamsted Experimental Station and reproduced in Table 1. Organic matter residues from six manuring treatments had been built up over several years in plots, with two sets of plots given inorganic fertilisers equivalent to two of the organic manures. From 1971 the plots had been in an arable rotation with inorganic fertilisers used on all plots. The experiment was arranged in two blocks, each containing eight plots to which the early manuring treatments had been applied. Each plot was divided into eight sub-plots to which a range of nitrogen dressing was applied. The tabulated values are the mean yields over the two blocks.

Examination of the data shows that for all manures the grain yield increased rapidly with increasing applied nitrogen to about 120 kg/ha, and then decreased rather erratically. Mean yields for the manuring treatments ranged from 4.26 to 5.59 t/ha, and the differences between manures were fairly constant over the range of applied nitrogen. The apparently simple conclusion that yield varied considerably with both manures and nitrogen levels, with little interaction, is belied by the analysis of variance given in Table 2.

Table 1 Yields of grain in tonnes/hectare from organic manuring experiment 79/W/RN/12

Nitrogen (kg/ha)	0	30	60	90	120	150	180	210	Mean
Manure									
FYM	1.82	3.82	5.77	6.06	6.88	5.64	6.18	5.89	5.26
Straw	1.72	3.90	5.48	6.68	6.42	6.58	6.48	6.00	5.41
Peat	1.38	3.83	5.52	6.42	6.50	7.13	5.89	6.21	5.36
Green manure	1.63	3.58	5.16	5.81	4.99	4.76	5.87	5.08	4.61
Fert=FYM	1.16	3.45	4.73	5.83	5.01	4.76	4.44	4.71	4.26
Fert=straw	1.81	3.69	5.44	6.49	6.87	6.33	5.99	5.81	5.30
Clover ley	2.29	4.28	6.11	6.55	6.98	6.17	6.17	6.13	5.59
Grass ley	1.57	3.60	5.19	6.52	6.25	5.79	6.52	6.29	5.22
Mean	1.67	3.77	5.42	6.29	6.24	5.90	5.94	5.77	5.13

Table 2 Analysis of variance of the yields of grain in experiment 79/W/RN/12

	df	MS	MSR
Blocks	1	23.78	130.66
Manures (whole plots)	7	3.23	0.88
Residual for whole plots	7	3.69	20.25
Nitrogen (subplots)	7	41.39	227.43
Manures × nitrogen	49	0.27	1.50
Residual for subplots	56	0.18	—

While the large and small mean square ratios for nitrogen and manures by nitrogen interaction respectively are as expected, the very small mean square ratio for manures is not. The simple conclusion from this analysis would be that yields for the various manures were closely similar. The clue to the contradiction between the analysis and common sense is in the very large mean square ratio for the whole plot residual compared with the subplot residual. One would expect whole plots to be the more variable but not to this extent. Only by examining the original data can the reason be found. For most manures the yields in block 2 exceed those in block 1 by less than 1 t/ha. The difference is a little larger for green manure, and considerably so for fertiliser equivalent to FYM (almost 3 t/ha). For grass ley there is a difference of about 1/4 t/ha in the opposite direction. It is this large variation in manure effects between blocks which inflates the whole plot residual mean square, and causes a difference in yield of about 1 1/2 t/ha to be declared statistically non-significant.

General interpretation

Overall, the interpretation of an analysis of variance is far from simple and is subject to a number of strong assumptions. Judicious inspection of tables of means may be much more informative. Standard errors provide a useful yardstick here but should not be allowed the status of automatic decision makers. One should beware also of the temptation to try to explain away every minor fluctuation in the data. This has led in the past to much unwarranted speculation and to the coining of meaningless phrases such as 'responsive sites' and 'crops able to utilise nitrogen efficiently'.

Subdivision of sums of squares

A common criticism of analysis of variance is that it fails to answer any specific questions. This is not a valid criticism of the technique, only of the experimenters for not posing specific questions. Any linear combination of levels of a factor may be extracted and tested if one wishes. The only provisos are that the questions generating the combinations should be based on features of interest to the experimenter and not as a result of inspection of the data, that multiple questions should not overlap, and that the number of questions that may be examined is, therefore, limited by the information available in the factor.

When the levels of the applied factor are quantitative, e.g. various rates of applied nitrogen, the detection of significant differences in yield does not imply

any particular functional form. Partitioning the sum of squares further into polynomial contrasts may indicate the existence of a general trend, simple curvature, or appreciable deviation from a smooth curve, but does not provide a useful working model for further study. The use of polynomials as approximations to other functions was valuable when computations had to be done by hand. With computers and good software now available it is possible to fit much more general functions. This is the basis of the predictive approach to the analysis of response data.

Predictive analysis

Here, the experimental data are used to estimate the values of the parameters in a model of chosen form. Ideally, the model would be based on a comprehensive theory of crop response, but it should at least accord with the known general form of response and be compatible with available theory. It should also be as simple as possible in form, and preferably be based on parameters with direct physical interpretation. Although one would naturally prefer a function which fits well to a particular set of data, it is more important that the model be capable of fitting reasonably well to a number of related sets of data with the same or a related set of parameters. In the long term it may be important that the model behave in a biologically sensible way when extrapolated but at the present stage of model development this is not crucial. However, given a choice between otherwise acceptable models with similar success in fitting to current data it is preferable to choose a model which is capable of generalisation with little change of functional form.

Review of models

Exponential equations

In a classic paper, Crowther and Yates (1941) reviewed all published results of fertiliser experiments from 1900 to 1940 in Great Britain and other northern European countries. The model used was the asymptotic exponential or Mitscherlich equation in the form

$$y = y_0 + d(1 - 10^{-kx})$$

where y is the predicted yield in tons/acre with nitrogen applied at the rate of x cwt/acre, y_0 is the yield with zero applied nitrogen, d is the asymptotic limit of yield and k is a constant.

Despite earlier criticisms of this model, they were apparently satisfied with its performance in this study but do not state how well the model fitted the data. The average value for the constant k is given as 1.1.

Bullen and Lessells (1957) used a similar technique on the results of a further 270 cereal experiments. They found that k varied from 0.13 to 1.68 for winter wheat, but that the average over experiments was again about 1.1. They were careful to point out that there was no question of this form of response curve

having the status of a universal law, but that the use of other curves is likely to be much less convenient in practice. Some of the force of this argument has now been lost with improvements in computing power but the law of diminishing returns aspect of the model is still attractive. The main shortcoming of the model is the lack of any descending portion at high levels of applied nitrogen. This may not have been a serious problem in these earlier experiments with relatively low levels of nitrogen dressing.

A common modification of the asymptotic exponential model is the addition of a linear term. The model is then usually written as

$$y = a + br^x + cx$$

where a , b , c and r are constant, $a > 0$ and $b, c < 0$; $0 < r < 1$.

Inverse polynomials

A totally different type of model was proposed by Nelder (1966). He argued that the response function should be capable of asymmetry, be non-negative and bounded, i.e. remain finite whatever level of applied nitrogen be considered. A family of models with these properties is the inverse polynomials of the general form

$$\frac{x}{y} = P_n(x)$$

where $P_n(x)$ is a polynomial of order n with non-negative coefficients. A problem arises in fitting such models due to the unknown amount of nitrogen available to the crop in addition to that applied. It is necessary to estimate this base level first and then to estimate the polynomial coefficients.

Inverse polynomials were proposed by Nelder as a convenient addition to the range of models, and have been shown to fit well to data from many sources. Inverse linear functions had been proposed earlier by Balmukand (1928). Greenwood et al., (1971) developed a rather simplified theory which led to a model of modified inverse linear form.

Split lines

Boyd (1972) noted that quadratic or exponential curves fitted to sugar beet data with five or six levels of applied nitrogen showed disturbingly similar residuals on different sites. He suggested that two straight lines would provide a good fit to many sets of experimental data. Anderson and Nelson (1975) took this idea further and allowed the possibility of three intersecting straight lines

Comparison of models

Boyd *et al.* (1976) compared a variety of models for the nitrogen response of cereals in 41 experiments and concluded that split lines performed best as judged by the average residual variances. Sparrow (1979) also compared a range of models for fitting to 83 spring barley experiments. The best model on the basis of

the residual mean square varied from one experiment to another but, overall, split lines were considered to be the best. It is interesting to note that he discarded two experiments because of the lack of any clear relationship between adjacent yields, but accepted all the other data as sufficiently reliable to provide an adequate comparison of models.

Multiple inputs

Some of the above single variable models may be generalised readily to cope with several nutrients, others have no simple generalisations. Since single nutrient response functions are not yet agreed it is premature to expect much progress with multi-dimensional functions.

The popularity of response surface designs in industry in the early 1960s prompted some interest in agricultural applications. The best known designs are 3^n factorials, possibly in fractional replication, and composite designs based on 2^n factorials with additional points for rotatability or to improve the accuracy of estimation in some directions. Inkson (1966) gave some examples and hinted at the use of sequential experimentation over several years. Since the optimum combination of nutrients may change considerably from year to year, and the designs concentrate on the fitting of quadratic surfaces, they are of limited value.

Yates (1966) briefly reviewed non-factorial designs and concluded that broader experimentation was still necessary.

Choice of model

General form of model

As demonstrated by the data in Table 1, experimental data over a sufficiently wide range of nitrogen dressing generally show a rapid increase followed by a slow decrease in yield with increasing applied nitrogen. Not all data follow this form. Some experiments show continuously increasing yields, others are continuously decreasing. Either of these is compatible with the more general form. More difficult to reconcile are experiments in which the yield fluctuates. These are often discarded as being unreliable or uninformative but they should not be dismissed too lightly. The fact that some experiments can produce such erratic results throws doubt on the reliability of others, and on the validity of discrimination between alternative models on the basis of small differences in the residual sums of squares.

Inspection of a large number of experiments showing both rising and descending portions of the response curve suggests that the variance is much larger at high levels of nitrogen than at low levels. The data given in Table 1, and plotted in Figure 1, are typical. The variance appears to increase sharply at, or perhaps a little below, the maximum value of the response curve. This is borne out by the variance components at the successive nitrogen levels, based on the differences between blocks adjusted for manure effects. The values obtained are 0.18, 0.05, 0.26, 0.19, 0.48, 0.36, 0.51 and 0.31; the ratio of the mean variance estimates for nitrogen levels of 120 to 210 and 0 to 90 kg/ha is 2.44. This is

approximately equal to the 1 per cent probability point for the F distribution with 28 and 28 degrees of freedom, so the difference is unlikely to have arisen by chance.

It seems likely that this increased variability at higher nitrogen levels is associated with the well-known increased risks of fungal attack and lodging. Both of these are catastrophic phenomena, i.e. even when conditions produce enhanced risk the crop sometimes escapes wholly or partly, but the affected part of the crop may suffer a serious reduction in yield. This sporadic loss of yield creates both an increase in variance and, more importantly, a net reduction of yield at the higher nitrogen levels. This not only distorts the shape of the response

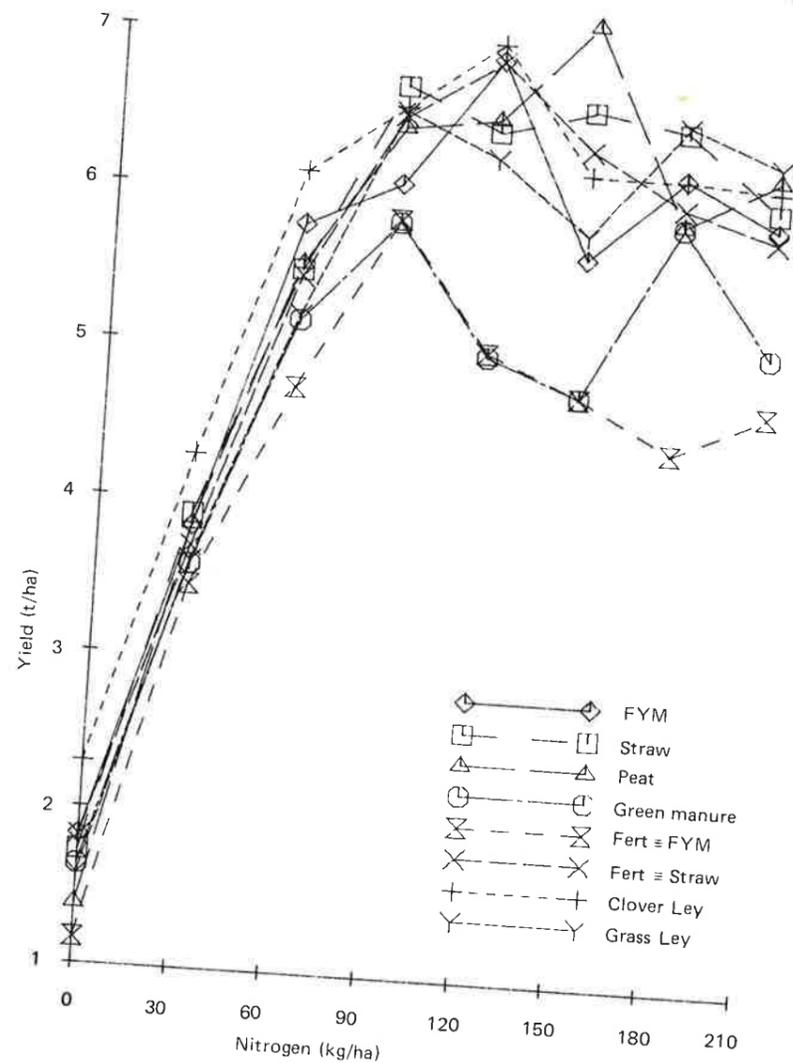


Fig. 1 Grain yields for the eight treatments in the long-term manuring experiment 79/W/RN/12.

function, to produce a sharp change of slope, but also poses a difficult problem in estimating the 'true' shape of the curve. The estimation method needs to be such that at lower levels of nitrogen positive and negative discrepancies about the fitted curve are equally likely, but at higher nitrogen levels low yield points are largely discounted in favour of a curve passing close to any high yield points. Although techniques exist to achieve this it is not easy to justify the use of such an asymmetric and irregular method. The difficulty may be eased by modern cereal varieties and the more widespread use of fungicides.

Previous experimentation

Many early experiments used only a few levels of applied nitrogen with the highest being too low to produce maximum yield. In addition, there was often inadequate control and recording of other factors, such as disease or level of other nutrients. More recent experiments have tried to span the maximum but have often been unduly complicated by the inclusion of too many other factors. In consequence, the exponents of the various models have been able to find data to support their cases, and no clear conclusion has been reached.

Two major red herrings have confused the issue. The first is the lack of recognition of the relative unreliability of data at higher nitrogen levels. The second is the emphasis on good numerical fit to individual sets of data, which both Yates (1966) and Nelder (1966) queried. In different ways, they stressed the need to seek confirmation from series of experiments. Nelder advocated the use of general parameters, with values remaining constant for experiments in similar conditions and varying in relation to changes in the conditions. The criterion of success of a model would then be that it fitted well to a series of related experiments, with changes in the parameters reflecting the differences between the experiments in the series. This criterion appears to have been overlooked by subsequent workers who have concentrated on producing the best possible fit to each individual experiment. As Nelder pointed out, a high value of R^2 , or equivalently a low residual mean square, in a single experiment is no guarantee of a good model.

A convenient working model

Wimble (1980), in a paper published after his death, described the four important features of a fertiliser response model as the slope of the rising part, the slope past the peak, the yield near the optimum dressing, and the location of the optimum dressing. To permit flexibility in matching four such characteristics a four parameter model is needed, and he listed the most popular models as the inverse quadratic, modified inverse linear, modified exponential and two straight lines. The latter model is unique in having a sharp break of slope and agrees well with many sets of experimental data. The break of slope found in the data may, however, be an indication of the onset of a limiting condition which may not remain in force in the future. The split-line model then accurately represents history but does not provide a basis for the estimation of the potential response of the crop. For this purpose, a smooth curve is preferable. The other three models

are all capable of representing the more general shape of a response curve, but the modified exponential has several advantages.

The law of diminishing returns interpretation of exponential curves has already been mentioned, and the addition of a linear term, with a negative coefficient, provides a simple way of allowing for reduced yields at higher nitrogen levels. Clearly, the linear term cannot be strictly valid since extrapolation of the curve will eventually produce negative values. In practice, few observations are normally made at nitrogen levels above the maximum and the linear term is a sufficiently good approximation, particularly in view of the doubtful reliability of these points.

With any multiparameter function, estimates of the parameters based on a moderate number of distinct nitrogen levels will be correlated. For the modified exponential model the estimates of c and r are quite highly correlated. This means that when the model is fitted to experimental data any divergence of one of these parameters from its optimum value may be largely offset by a corresponding change in the value of the other. Since the major practical difficulty in fitting the model is the estimation of the non-linear parameter r , it would be advantageous if this could be fixed at some value close to its optimum. Fitting the model would then be very simple since all terms would be linear. Determination of the optimum value of r for a number of cereal experiments gave estimates varying from about 0.985 to just over 0.99. Varying the value of r from 0.98 to 0.995, and estimating the best corresponding values of a , b and c , was found to produce negligibly different values of R^2 for individual experiments. It has subsequently been found that fixing r at 0.99 allows a wide range of experiments to be adequately fitted. It is of interest to note that the value of $k = 1.1$ found by Crowther and Yates is equivalent to $r = 0.98$, allowing for the change in the units of measurement.

The remaining two parameters in the modified exponential model, a and b , correspond essentially to the vertical and horizontal location of the curve. If two curves, with the same value of c , have the equations

$$y_1 = a_1 + b_1 r^x + cx \text{ and } y_2 = a_2 + b_2 r^x + cx$$

the second may be rewritten as

$$y_2 = a_1 + \Delta a + b_1 r^{x+\Delta x} + c(x + \Delta x)$$

$$\text{where } \Delta x = \ln(b_2/b_1)/\ln(r) \text{ and } \Delta a = a_2 - a_1 - c\Delta x$$

The second equation is then seen as being the same as the first, but with a horizontal shift of Δx and a vertical shift of Δa . As response curves, the interpretation is that the second function has a maximum yield Δa units higher than the first, attained at an applied nitrogen level Δx units higher. The principle extends to more than two curves. If a family of curves be fitted to a series of related experiments, with c held constant, but estimated from the data, and both a and b allowed to vary from experiment to experiment, the differences between the experiments may be summarised in simple terms as the differences in maximum yield and the differences in nitrogen level at which the maxima are attained.

Families of curves have been fitted in this way to several series of experiments. Details will be published elsewhere, but the percentage variation explained over series of 30 to 40 experiments has generally been about 95 to 99 per cent. This is not perhaps as impressive as it might appear since it is usually possible to account for 50 to 60 per cent of the variation by fitting a single curve to the whole series.

In some cases it has been possible to find simple interpretations of the vertical and horizontal shifts. In a series of experiments with four cropping sequences over nine years, differences in yield were related largely to years while differences in nitrogen level were related largely to cropping sequences.

The maximum of the modified exponential curve occurs when

$$x = \ln \left\{ -c/[b \ln(r)] \right\} / \ln(r).$$

Although easily calculated the value is not very reliable since the curve is relatively flat around the maximum.

The economic optimum for a price ratio of $p:1$ is at

$$x = \ln \left\{ (p-c)/[b \ln(r)] \right\} / \ln(r).$$

Since c is generally small (about -0.01 to -0.03) and p is constant at about 0.003 to 0.004 (with the grain yield measured in t/ha and nitrogen application in kg/ha), this is determined largely by the value of c/b .

One useful mathematical property of modified exponential curves with a constant value for r is that the average of two curves is of the same form. If c is also constant, the x value at the maximum of the average of two such curves is as above but with b replaced by the average value \bar{b} . The average of the x values at the two separate maxima is again as above but with b replaced by the geometric mean $\sqrt{b_1 b_2}$, with the appropriate sign. If the separate values of b are similar, the simple average and the geometric mean will be little different; e.g. if $b_1 = -9$ and $b_2 = -10$, then $\bar{b} = -9.5$ and $\sqrt{b_1 b_2} = -9.487$. Many experiments are replicated in blocks and the curve is then fitted to the average values of yield over the blocks. Although the yields may differ appreciably between blocks, the nitrogen level at the maximum is generally almost constant. The fitted curve then estimates the optimum nitrogen dressing accurately, and the differences between the blocks provides a valid estimate of experimental error.

When two or more sets of data differ appreciably in location their average is of no interest. If the shape of the curves may be assumed to be the same, fitting a family of curves differing only in the values of a and b allows the fitted curves to be superimposed by suitable horizontal and vertical shifts. The deviations of the adjusted data about this common curve are then the appropriate measure of experimental error. Fitting a family of curves in this way to the data in Table 1, with $r = 0.99$, gave an estimated common value of $c = -0.33$ and the values of a and b given in Table 3. The calculated maximum yields and corresponding nitrogen levels are also tabulated, together with the horizontal and vertical shifts required to bring the fitted curves into coincidence with that for FYM. The adjusted data are plotted in Figure 2, where the values marked on the axes are those for FYM. The good fit of the common curve at low levels of applied nitrogen, and the general increase in variability at higher levels, are easily seen.

Table 3 Fitted parameters and differences in yield and N level for data in Table 1

Manure	a	b	Y Max	N Max	ΔY	ΔN
FYM	14.030	-12.196	6.47	131.3	—	—
Straw	14.405	-12.710	6.71	135.4	0.24	4.1
Peat	14.512	-13.065	6.73	138.1	0.26	6.8
Green manure	13.017	-11.361	5.69	124.2	-0.78	-7.1
Fert=FYM	12.567	-11.129	5.30	122.2	-1.17	-9.1
Fert=straw	14.185	-12.446	6.56	133.3	0.09	2.0
Clover ley	14.209	-11.857	6.74	128.5	0.27	-2.8
Grass ley	14.286	-12.877	6.55	136.7	0.08	5.4

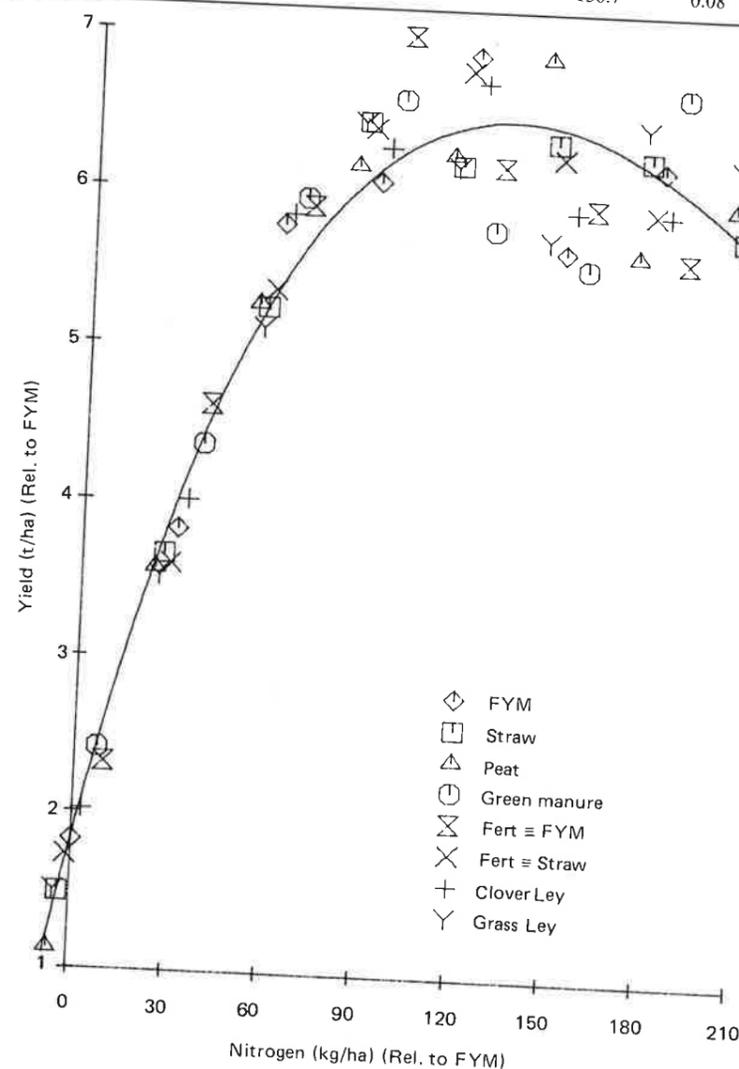


Fig. 2 Grain yields in experiment 79/W/RN/12 adjusted relative to FYM to bring the fitted curves into superposition.

In this example the maximum yields are all attained at similar applied nitrogen levels. In other cases this may not be so and the gradient of the response function at zero applied nitrogen may be appreciably different from one treatment to another. Description of response in terms of the rate of increase in yield between zero applied nitrogen and the maximum, or the yield at some arbitrarily chosen level of applied nitrogen, will reflect this gradient. It will indicate the response per unit applied nitrogen at low levels but this is a combination of the nitrogen available in the site and the shape of the response curve for the particular crop. What is really required is the separation of these two terms, as afforded by the estimation of the maximum yield and the level of nitrogen application at which this is attained.

Development of the model

Although the modified exponential curve is mathematically convenient and provides a good fit to many series of experiments, the model is only a first approximation to biological reality. Extrapolation in either direction produces negative yield values. A more realistic model would level off to zero yield at low levels of nitrogen (negative values of applied nitrogen), and would also level off at high levels of nitrogen to a possibly non-zero yield. A possible function with these characteristics might be the modified logistic

$$y = \frac{a + br_1^x}{1 + cr_2^x}$$

where a, b and c are all positive, and r_1 and r_2 both lie between 0 and 1. For low and moderate levels of x this will be dominated by the denominator, and at high values of x by the numerator. For suitable values of the parameters it can be closely approximated by a modified exponential function over a wide range of values of x.

The modified logistic model contains five parameters. To determine these, or a similar set of parameters for other general models, would require experimentation at very low levels of nitrogen, obtainable only on seriously impoverished sites, and at very high levels of applied nitrogen on similar sites. The results might be of academic interest but would have little practical value.

Experimental requirements

Single experiment

In order to fit a four parameter model it is clearly necessary to have at least four data points. With only four points the fitted model may pass through them precisely. With only a few additional independent points there will be little information available to determine the variability about the fitted line, and it will be quite impossible to recognise whether this variability is random or systematic. The least squares principle normally used to estimate the parameters will tend to

produce similar positive and negative errors about the fitted curve. Only if these show a distinct pattern, for example positive for high and low nitrogen levels and negative for intermediate levels, will there be any suggestion of lack of fit, and even then it will be only an unprovable suggestion. To fit and adequately test a model many points are needed, with replicate observations at some or all of a number of distinct nitrogen levels, which must be appreciably more than four.

With n replicate observations at k nitrogen levels, the best fitting line will be determined effectively by the averages of the k groups of yields. The deviations of these averages from the line will provide a measure of the lack of fit of the model, and the deviations of individual yields from their corresponding averages will provide a measure of pure error. The ratio of the mean squares for lack of fit to that for pure error may be compared with the appropriate F distribution to test if the lack of fit is significantly large. If not so, the model is an adequate fit to the data.

Since four parameters have to be estimated, the lack of fit mean square has $\nu_1 = k - 4$ degrees of freedom. The pure error mean square has $\nu_2 = k(n - 1)$ degrees of freedom. The F test is more sensitive to changes in ν_2 than in ν_1 , and ν_2 should be reasonably large. As a general rule of thumb one should aim for $\nu_2 = 12$, and $\nu_1 = 3$. Reasonable values for this single experiment would then be $k = 7$ and $n = 3$, so that $\nu_1 = 3$ and $\nu_2 = 14$.

It is not necessary to have the same number of replicates at each nitrogen level. With n_1, n_2, \dots replicates at the various levels, $\nu_2 = \sum (n_i - 1)$. This gives a little more freedom, and one might choose to set $k = 8$ and allow n_i to alternate between 2 and 3, to obtain $\nu_1 = 4$ and $\nu_2 = 12$, from a total of 20 experimental units.

The optimum spacing of the nitrogen levels is less easily defined. There is a natural tendency to use equally spaced levels but this is not necessary and is certainly not optimum for a non-linear model. In theory, for any assumed form of model it would be possible to calculate the optimum spacing but this would involve some further assumption such as the position of the maximum. The computations would be laborious and the gain in precision over the use of equally spaced levels would probably be quite small.

The lowest possible value of applied nitrogen is, of course, zero. Since the precision of the parameter estimates increases with the increasing range of nitrogen levels it is clearly advantageous to set the lowest level of application at zero, but this does not imply that this provides any special information. It is only the zero level of applied nitrogen. The actual nitrogen level will depend on the site. Since there is evidence that the grain yield is erratic at nitrogen levels much above the maximum yield value, the mathematical advantage of setting the highest level of applied nitrogen as high as possible may be offset by a breakdown of the model. The practical limit is, therefore, only a little above the expected maximum yield point.

The major determinants of the nitrogen levels to be used in an experiment are practical rather than theoretical, but some advantage may be taken of the relative stability of the response at lower nitrogen levels. Few points are required on the steeply rising part of the curve, compared with the number around the maximum.

Although the descending portion of the curve is of little practical interest, the relative instability of the yields here requires a disproportionate number of points, or replicates, to avoid the risk of serious error in the parameter estimates.

The shape of the fitted curve will be particularly sensitive to the yields at these high levels of nitrogen.

Series of experiments

When data are available from a series of related experiments, or possibly when a range of nitrogen dressings has been applied at several levels of some other factor, it may be reasonable to assume that the shape of the response function is the same for each experiment in the series. Only two independent parameters are then required for each individual experiment, with two further general parameters. Sufficient degrees of freedom to test the fit of the model adequately may then be obtained with much smaller numbers of both applied nitrogen levels and replicates.

If, in a series of s experiments, each experiment has k nitrogen levels and n replicates of each level, the degrees of freedom for fitted parameters, lack of fit and pure error are $2(s + 1)$, $sk - 2(s + 1)$ and $sk(n - 1)$ respectively. With $s = 4$ it would be possible, but not advisable, to have $k = 4$ and $n = 3$, giving 6 and 32 degrees of freedom for lack of fit and pure error. A better arrangement for the same total number of experimental units would be $k = 6$ and $n = 2$, giving 14 and 24 degrees of freedom.

As in the single experiment, it is not necessary to have equal replication of each nitrogen level. In addition, it is not necessary to have the same nitrogen levels in a series. This allows considerable flexibility in arranging the experiments. Provided that the assumption of constant shape were secure, it would be possible to include some experiments in the series with very few experimental points. Estimates of lack of fit and error would then rely on the more intensive experiments in the series.

The strong dependence on the assumption of constant shape is reduced if the experiments in the series are similar and each contains enough distinct nitrogen levels to be capable of fitting a curve independently with some degrees of freedom left for estimating the lack of fit. The lack of fit term in the composite analysis can then be subdivided into components representing the lack of fit in each individual experiment and the overall lack of fit in the series. In the above example with $k = 6$ and $n = 2$, the 14 degrees of freedom for lack of fit may be partitioned into 8 degrees of freedom (two from each of the four experiments) for individual experiments and 6 degrees of freedom for overall lack of fit. The alternative arrangement with $k = 4$ and $n = 3$ supplies only the 6 degrees of freedom for overall fit, i.e. for general agreement of the model with the data. It does not provide any means of testing the differences in fit for the individual experiments.

Conclusions

The response of cereal yields to applied nitrogen generally follows a steeply rising followed by a less steeply falling curve. Extra sources of variation apply at higher nitrogen levels causing erratic results and decreased yields. This may have misled previous workers into proposing response functions with sharp breaks of slope. The modified exponential function, having a linear term with negative

coefficient, has been found to fit reasonably well to many sets of data. The exponential term appears to differ little in a range of experiments and may be fixed at $r = 0.99$, for nitrogen applications in kg/ha and yields in t/ha, with little loss of generality. The modified exponential function is particularly convenient for fitting to series of experiments when the response function is roughly constant in shape but varies in location. A possible underlying model is the modified logistic function. Current experimental data is inadequate to test this. A curve fitting approach to the examination of response data allows much more flexibility than does an analysis of variance approach, and does not contain the same implication of constant horizontal location of the response function. In addition, it allows the response function to be described in terms of a limited number of parameters with possible biological interpretation. Differences between fitted response functions for experiments in a series may be expressed simply in terms of the maximum yield and the corresponding level of applied nitrogen, or similar quantities for any required economic yield criterion. Series of experiments intended to be examined by curve fitting may be arranged in a flexible way. Ideally, each experiment should be capable of analysis independently, should span the expected maximum for the site, and should contain direct replication of some applied nitrogen levels. The residual error term may then be partitioned to provide tests of lack of fit of the model.

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Discussion

Dr Hughes said that the 'catastrophic zone' was evident in many of the results of the recent cereal trials. He asked if there was more replication within each block, would this show that the points above the curve were subject to a greater degree of error. Mr George replied that catastrophes would occur on all replicates. However, with more replication of the high nitrogen treatments it would be possible to estimate variance directly. Unfortunately the higher nitrogen treatments were unstable figures, and in excess of the general nitrogen recommendations, but it would be desirable to have replication of the high rates within each block.

Dr Jenkinson asked if there were different types of catastrophe, for example, pests or lodging. He also asked if local variations in soil fertility were important. Mr George replied that it was unlikely that catastrophe was due to different levels of fertility within a trial site. Catastrophe or erratic behaviour is not noticeable on the lower part of the response curve. Erratic behaviour is only noticed on the part of the curve above the optimum, and therefore unlikely to be due to changes in soil fertility.

Dr Batey asked if the same sort of catastrophe occurred in other crops, for example grass where only vegetative growth was measured. The lower part of the curve was associated with vegetative growth and therefore a direct response to nitrogen. Mr George replied that he had no experience as to the response of grass to nitrogen. However, the results quoted in the paper were part of a rotational experiment, involving sugar beet, potatoes and spring barley. The sugar beet showed similar response characteristics to wheat.

Mr Lidgate asked if catastrophe was too strong a word. He also stated that on the part of the curve above the optimum, nitrogen was no longer the limiting factor to yield. Mr George replied that he has tried to define catastrophe as a naturally occurring phenomenon, not as a disaster.

Dr Tinker asked if the response curve to other nutrients had been studied. Mr George replied that as yet he had not studied the effect of other nutrients and obviously this required further work.

Mr Whitear said that in an analysis of variance each plot contributes an equal amount of error. From this approach, did the amount of error increase at higher yields. Mr George said that the changes in variance were not smooth. Values for variance were quoted in the paper, and there was an abrupt change in the variance at or above the optimum nitrogen level. The variance values were much less at the lower nitrogen rates.

Mr Wadsworth asked if shapes of the response curve would be same from trials carried out on apparently similar sites, where there were differences in both yield and optimum nitrogen level. Mr George replied that if experiments were conducted on similar sites then one would expect similar shapes in the response curve.