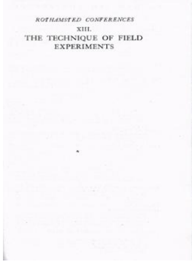
XIII. The Technique of Field Experiments


ROTHAMSTED CONFERENCES
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Methods of Field Experimentation and the Statistical Analysis of the Results

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namely that it is necessary that our methods of arrangement in the field must be brought rigorously into harmony with the methods of computation to be employed. For given methods of arrangement it is possible that there shall be at most one correct method of computation, and this one we must be able to recognise and to use. For many methods of arrangement, however, no method of estimating the error, which is strictly valid, can possibly exist.

It is thus seen that the second object of replication, the diminution of error, may, if a sufficient number of plots can be used, be carried to any required degree of precision, at least if the primary principle of replication is supplemented by the principle of Local Control. With respect to the first object of replication—to provide an estimate of error—we must now note that, if we are to obtain a strictly valid estimate of error, then it is necessary, in order to satisfy the mathematical conditions on which the use of such an estimate is based, that, apart from such restrictions as are introduced in the complete elimination of certain components of the soil heterogeneity, the different treatments or varieties to be tested shall be arranged at random on the land available. One may say that the heterogeneity of the experimental land is in this way divided into two parts, one of which is totally eliminated from the experiment by the field arrangement, and subsequently in the arithmetical procedure, while the other part is scrupulously randomised in the field arrangement, in order that that portion of it which will be available for the estimation of error shall be truly representative of that portion which necessarily will appear as real errors in our results. The methods by which these principles of experimentation have been worked out in detail are very various, and several examples of these will be given by later speakers.

METHODS OF FIELD EXPERIMENTATION AND THE STATISTICAL ANALYSIS OF THE RESULTS

By JOHN WISHART

The two simplest methods of layout which fulfil the conditions of supplying a valid estimate of error and eliminating a large portion of the soil heterogeneity are (1) the method of Randomised Blocks, and (2) the method of the Latin Square. In what follows these methods will be described shortly, with appropriate arithmetical illustrations to indicate how the results of such trials should be analysed.

(1) Method of Randomised Blocks

As an example consider an experiment carried out in 1930 by the Rothamsted staff at the farm of Mr. E. V. Cooke, North Fen, Bourne (Lincs.). The crop was potatoes, and the treatments tested were: superphosphate at the rate of 0, 0·8 and 1·6 cwt. P_2O_5 per acre (approximately 5 and 10 cwt. superphosphate), and sulphate of potash at the rate of 0, 1 and 2 cwt. K_2O per acre (approximately 2 and 4 cwt. sulphate of potash), in all combinations. There were thus nine treatments in all, and these were laid down in four-fold replication. A plan of the experiment is shown below. The area was divided into four equal blocks, each consisting of nine plots, and the nine treatments were allotted at random to the plots within each block. The plot yields, in lb., are given in the table:—

A			B		
0S 2K	1S 2K	1S 1K	0S 1K	0S 2K	1S 1K
2S 1K	0S 1K	2S 0K	2S 0K	1S 0K	2S 1K
1S 0K	2S 2K	0S 0K	1S 2K	2S 2K	0S 0K
1S 1K	0S 2K	2S 2K	1S 0K	2S 0K	0S 2K
0S 0K	1S 0K	1S 2K	1S 1K	2S 2K	1S 2K
2S 1K	2S 0K	0S 1K	0S 0K	0S 1K	2S 1K
C			D		

Area of each plot = 1/70 acre.

0S = No super.

1S = 0·8 cwt. P_2O_5 as super.

2S = 1·6 cwt. P_2O_5 as super.

0K = No potash.

1K = 1 cwt. K_2O as Sul./Pot.

2K = 2 cwt. K_2O as Sul./Pot.

Block	*No Potash*			*Single Potash*			*Double Potash*			Total
	0S	1S	2S	0S	1S	2S	0S	1S	2S	
A	372	293	392	360	459	388	344	439	406	3453
B	334	444	437	393	385	434	366	438	439	3670
C	234	291	279	295	339	297	332	413	479	2959
D	262	385	338	335	382	367	297	365	421	3152
Total	1202	1413	1446	1383	1565	1486	1339	1655	1745	13234
							General Mean = 367·61			

The block and treatment totals are given in the margins of the above table, while the general mean of all plots is 367·61. We now regard the deviation from this general mean of each plot yield as being made up in the main of two parts, one equal to the deviation of the block mean from the general mean, and representing the amount by which the yield is influenced by the fertility of the block in which it happens to be situated, and the other equal to the deviation of the mean of the treatment given to that plot from the general mean, and measuring the value of the particular treatment as far as this plot is concerned. The sum of these two portions gives a theoretical value for the plot yield deviation, and the differences, positive and negative, from this theoretical value, of the deviation of the actual plot yield from the general mean of all plots, represent the errors of the experiment, and are used to furnish a standard error for the treatment means, or totals. The sum of squares of these residuals is best obtained by eliminating the contributions due respectively to blocks and treatments from the total sum of squares of deviations of the 36 plot values from the general mean. Instead of subtracting the mean successively from each value, and squaring and adding the remainders, it is only necessary to square and add the actual values as given in the table, and then subtract a correction equal to the square of the grand total divided by 36. The same result could be secured, but with smaller numbers to work with, by subtracting a round number near to the true mean, as, for example, 400, from all the values before squaring. The correction in this case is the square of the new total, divided by 36. The arithmetical working is as follows :—

Sum of squares of 36 plot yields . .	4994120	
Grand total squared and divided by 36 .	4864965·44	
Difference	129154·56	(*a*)
Sum of squares of 9 treatment totals . .	19679370	
Divide by 4 (since each is a total of 4 plots) .	4919842·5	
Subtract as above	4864965·44	
Remainder	54877·06	(*b*)
Sum of squares of 4 block totals . .	44082894	
Divide by 9 (since each is a total of 9 plots) .	4898099·33	
Subtract as above	4864965·44	
	33133·89	(*c*)
(*a*) — (*b*) — (*c*)	41143·61	(*d*)

The sums of squares (*b*), (*c*) and (*d*) must now be divided by the appropriate numbers of degrees of freedom to obtain the corresponding mean squares. There are 35 degrees of freedom in all (one less than the total number of plots), of these there are 8 for treatments (one less than the total number of treatments) and 3 for blocks (one less than the total number of blocks), so that there are 24 remaining for the error part. The results are now set out in the form of an "analysis of variance" table as follows:—

Analysis of Variance due to	*Degrees of Freedom*	*Sums of Squares*	*Mean Square*
Blocks	3	33133·89	11044·63
Treatments	8	54877·06	6859·63
Error	24	41143·61	1714·32
Total	35	129154·56	

We note that the method of arrangement has removed, under the heading "blocks," a substantial amount of the soil heterogeneity present. Had the arrangement not been such as to allow for this, the error "mean square" would have been very much higher. The next point to note is that the treatment "mean square" is materially greater than that due to error. With no real treatment differences, these two contributions should have been equal within the limits of sampling error, and we test whether the treatment mean square is significantly greater than the error mean square by finding the ratio of these two quantities, *i.e.* 4·001, and then determining one-half the natural logarithm of the ratio. This gives us the quantity z, which is in this case equal to 0·6932. The same result is obtained by finding the ordinary logarithm of 4·001, *i.e.* 0·6022, and multiplying by 1·1513. From R. A. Fisher's "Statistical Methods for Research Workers," table of z, we find from n_1 (the number of degrees of freedom corresponding to the larger mean square) $= 8$, and n_2 (the number of degrees of freedom corresponding to the smaller mean square) $= 24$, a z value of 0·6064 for the 1 per cent. point. Thus the value 0·6064 would be exceeded by chance only once in a hundred trials, had there been no real treatment differences, and we therefore conclude that as the actual z is 0·6932 the effect of treatment is undoubtedly significant. It remains now to examine the nature of this treatment effect. The square root of the error mean square (1714·32) is 41·40, and this is the estimated standard error of a single plot. The standard error of a total of four plots is got by multiplying this by the square root of 4, and is therefore 82·8, while the standard error of a total of 12 plots is got by multiplying by the square root of 12, and is 143·4. We may therefore rearrange our treatment totals in the following way, in order to bring out the real effects, and we append the appropriate standard errors.

TOTALS OF FOUR PLOTS

	No Super	*Single Super*	*Double Super*	*Total*
No Potash .	1202	1413	1446	4061
Single Potash	1383	1565	1486	4434
Double Potash	1339	1655	1745	4739
Total	3924	4633	4677	13,234

Standard error, 82·8 or 5·63 per cent. ; of margins, 143·4 or 3·25 per cent.

The difference between any two totals should exceed three times the standard error for significance, and an inspection of the table shows that the main effects are a response to superphosphate which is not increased materially by the double dressing, and a response to sulphate of potash which is of such a nature that the yield from the single dressing is intermediate between the other two, although alone the increase is barely significant over the yield of the plots without potash. These results are gleaned from the margins of the above table, which do something to smooth out the irregularities of the individual figures, and which are based on the means of 12 plots. The only additional effect that could emerge would be an interaction between superphosphate and sulphate of potash, *i.e.* where the increase on a plot having both was something more than the sum of the separate increases due to the single nutrients. There is not much sign of such an interaction in the individual figures of the table, but these treatment effects can be tested more precisely, since the experiment is of a balanced type, by breaking up the total sum of squares due to treatment into two parts due respectively to superphosphate and potash, and a third part representing the interaction. The calculations are similar to those already carried through for blocks and treatments, and are as follows :—

Sum of squares of 3 super totals .	58736794
Divide by 12 (since each is a total of 12 plots) . . .	4894732·83
Subtract as in previous work .	4864965·44
	29767·39 (2 degrees of freedom)

Sum of squares of 3 potash totals	58610198	
Divide by 12	4884183·17	
Subtract as above . . .	4864965·44	
	19217·73	(2 degrees of freedom)
Remainder on subtracting from 54877·06	5891·94	(4 degrees of freedom)

The central part of our analysis of variance table may then be given more fully as follows :—

Due to	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	
Super. . .	2	29767·39	14883·70	$z = 1{\cdot}0806$
Potash . .	2	19217·73	9608·86	$z = 0{\cdot}8618$
Interaction .	4	5891·94	1472·98	
[Error . .	24		1714·32]	

z for 1 per cent. point ($n_1 = 2$, $n_2 = 24$) = 0·8626

It is obvious that the interaction is wholly insignificant, the mean square being nearly equal to that for error, while by testing the superphosphate and potash effects by the z test with $n_1 = 2$ and $n_2 = 24$, it appears that both effects are significant. We see therefore that the method of arrangement has not only given us effectively greater replication for the potash and superphosphate comparisons, but has shown that the effects demonstrated for each treatment hold, within the limits of experimental error, over a wide range of the other fertiliser. The analysis is completed by presenting the data of our table in agricultural units, *e.g.* tons per acre. Since the yields given in the body of the table are in lb. per $^4/_{70}$ acre, we must divide them, and the standard error of 82·8, by $^4/_{70} \times 2240$, or 128, while the marginal totals, and the standard error of 143·4, are to be divided by 384 to give marginal means in tons per acre.

(2) Method of the Latin Square

We shall take as an illustration of this method a trial carried out on sugar beet at the South-Eastern Agricultural College, Wye, in 1930. The treatments were (1) control, (2) sulphate of ammonia (3 cwt. per acre) applied with seed, (3) nitrate of soda (equivalent to 3 cwt. sulphate of ammonia) as top dressing, and (4) cyanamide (equivalent to 3 cwt. sulphate of ammonia) applied a few days before sowing. There were four replications of each treatment, and the plots were arranged as in the diagram, so that each treatment occurred once and once only in each row and column of the Latin square. The particular arrangement adopted was one chosen at random out of the total number of Latin squares of this size.

I	S	N	C	O
II.	C	S	O	N
III	O	C	N	S
IV	N	O	S	C

Area of each plot = 1/50 acre.
O = control.
S = sulphate of ammonia.
N = nitrate of soda.
C = cyanamide.

All plots received 4 cwt. superphosphate and 2 cwt. muriate of potash per acre.

YIELD OF UNWASHED ROOTS IN LB.

	Columns				*Total*
Rows	654	661	673	599	2587
	638	621	573	719	2551
	499	581	639	652	2371
	557	477	591	669	2294
Total	2348	2340	2476	2639	9803

General mean, 612·6875

TREATMENT TOTALS

O	S	N	C	*Mean*	*Standard Error*
2148	2518	2576	2561	2450·75	37·92

With this method of arrangement, the rows of plots are replicates of one another, and equally the columns, and a comparison of their totals measures the effect of soil heterogeneity, which can be eliminated in the calculation of the standard error of the treatment means. The deviation from the general mean of the yield of any one plot is in fact regarded as made up in the main of three parts, one equal to the deviation of the row mean from the general mean, another equal to the deviation of the column mean from the general mean, and the third equal to the deviation of the treatment mean from the general mean. The sum of squares of the residuals, or differences of the actual yield deviations from theoretical values made up of these three parts, is then used to furnish an estimate of the required standard error. As in (1) this sum of squares is best obtained by eliminating the contributions due to rows, columns and treatment from the total sum of squares of deviations of the 16 plot values from the general mean. The arithmetical working is as below :—

Sum of squares of 16 plot yields	6068949		
Grand total squared and divided by 16 . .	6006175·56		
Difference	62773·44	(*a*)	(15 degrees of freedom)
Sum of squares of 4 row totals	24084247		
Divide by 4 (since each is a total of 4 plots) . .	6021061·75		
Subtract as above . .	6006175·56		
Remainder	14886·19	(*b*)	(3 degrees of freedom)
Sum of squares of 4 column totals	24083601		
Divide by 4 (since each is a total of 4 plots) . .	6020900·25		
Subtract as above . .	6006175·56		
Remainder	14724·69	(*c*)	(3 degrees of freedom)
Sum of squares of 4 treatment totals	24148725		
Divide by 4 (since each is a total of 4 plots) . .	6037181·25		
Subtract as above . .	6006175·56		
Remainder	31005·69	(*d*)	(3 degrees of freedom)
(*a*) — (*b*) — (*c*) — (*d*) . .	2156·87		(6 degrees of freedom)

Analysis of Variance due to	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	
Rows . .	3	14886·19	4962·06	
Columns .	3	14724·69	4908·23	
Treatments .	3	31005·69	10335·23	$z = 1{\cdot}6794$
Error . .	6	2156·87	359·48	
Total	15	62773·44		

The significance of the treatment differences is here unquestioned, since the 1 per cent. value of z for $n_1 = 3$ and $n_2 = 6$ is 1·1401, and the value reached, 1·6794, exceeds this. The standard error of a total of four plots is

$$\sqrt{(359 \cdot 48 \times 4)} = 37 \cdot 92, \text{ or } 1 \cdot 55 \text{ per cent. of the mean.}$$

It is clear from this standard error, taken in conjunction with the table of treatment totals given earlier, that the result of the experiment may be summarised by saying that there was a significant increase in yield due to the application of nitrogeneous fertiliser, whatever the form, but that there were no differences in action between the forms of fertiliser, or between the times at which these were applied.

The two experiments selected in this paper are an illustration of the fact that the Latin square method usually provides greater accuracy of comparison (here 1·55 per cent. as against 3·25 per cent. at the lowest). This method is in fact the best where the number of treatments is not too great, *e.g.* up to six or seven, and particularly where all comparisons made are of equal value, as in testing equivalent quantities of the same fertiliser, or in testing different varieties. For experiments involving larger numbers of treatments, as in all cases of the balanced type illustrated in (1), the randomised block method is suitable, but care should be taken that a sufficient degree of replication is provided for.

A brief statement only has been given of the methods of laying out and analysing experimental trials, and each experiment treated usually produces points of its own for consideration, while experiments of a more complex nature than those dealt with here can also be readily carried out. For a more detailed discussion of the principles of the method, and of the method of analysis, the following references should be consulted :—

R. A. Fisher : "Statistical Methods for Research Workers," 3rd edn., 1930, Chapters VII and VIII. Oliver & Boyd. 15s.

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