A PROGRAM FOR SPLIT PLOT ANALYSIS OF VARIANCE USING SMALL, PROGRAMABLE CALCULATORS

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Many field experiments conducted by plant pathologists and entomologists can be analyzed in minutes without knowledge of the computations involved or the use of large scale computer-data processing facilities which are often inefficient of both time and cost for small statistical tests. The Texas Instruments SR-52 hand held programable calculator provides an economical, rapid, and simple means for data analysis from field experiments. A description of this calculator and a practical program for a two-way analysis of variance of a randomized complete block designed experiment was given in Insecticide and Acaricide Tests, 2:7-10. The purpose of this article is to describe a program for the split plot analysis of variance in randomized complete blocks.

The split plot design offers a great deal of flexibility and efficiency in many field tests. For example, consider an experiment in which crop varieties are tested for their performance in pathogen-infested field soil. A two-way analysis of variance in randomized complete blocks might entail 4 to 6 replications of each variety arranged such that the varieties are randomly assigned within each replicate. Using a split plot design, an additional factor, chemical seed protectants, may be included in the experiment. Each variety becomes a 'whole plot' that is subdivided to include each seed protectant. The seed protectants, or 'subplots', are randomized within each whole plot. Randomization is two-stage: varieties are randomized within replicates and seed protectants are randomized within varieties. A complete review of this design may be found in Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company. New York. 481 pages, and other textbooks on statistical analysis.

The split plot program for the Texas Instruments SR-52 calculator is contained on three separate program cards. Individual data are entered on Card I, subplot means obtained during Card I program operation are entered on Card II, and machine calculations are completed on Card III, giving a direct display of appropriate F-tests and coefficients of variability. The program allows a maximum of six replications and an unlimited number of levels of whole plots (Factor A) and subplots (Factor B). The 'Users Directions', a sample analysis of variance table, formulas for calculations of standard errors of the mean for use in the Least Significant Difference Tests, and program sequence are given below.

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'USERS DIRECTIONS'

1) **Insert CARD I both ways to program calculator. (See owner's manual).**

2) **Enter data by subplots across replicates. (Enter rep 1, rep 2, etc. at the same level of Factor B). PRESS A.**

3) **Obtain the mean for the level of Factor B. PRESS B. Record means for use later.**

4) Repeat steps 2 and 3 for each level of Factor B within the first level of Factor A.

5) When all levels of Factor B have been entered in the first level of Factor A, PRESS C. (Display will show the number of levels of Factor A entered).

6) Repeat steps 2 through 5 for each level of Factor A.

7) When all levels of Factor A have been entered, PRESS RUN.

8) Insert CARD II both ways. (See owner's manual).

9) PRESS 2nd A'. (This starts the calculations).

10) Enter the means of the first level of Factor B across levels of Factor A. PRESS A. (The means were obtained from step 3).

11) When all means have been entered for the same level of Factor B, PRESS B.

12) Repeat steps 10 and 11 for each level of Factor B.

13) When all means have been entered, PRESS C.

14) Use memory recall to obtain sums of squares (S.S.) by PRESSING RCL _ _. (The contents of the memory registers are given below).

15) Insert CARD III both ways. (See owner's manual).

16) PRESS 2nd A'. (This starts the calculations).

17) Obtain F value for replications. PRESS A. (An F value is declared significant if it is as large or larger than the tabular F from statistical tables --- this comparison is based on the degrees of freedom of the appropriate error term and the effect being tested).

18) Obtain F value for Factor A. PRESS B.

19) Obtain F value for Factor B. PRESS C.

20) Obtain F value for Interaction A x B. PRESS D.

21) Obtain coefficient of variability (C.V.) for the whole plot analysis (Factor A). PRESS E.

22) Obtain coefficient of variability (C.V.) for the subplot analysis (Factor B). PRESS 2nd E'.

23) Use memory recall to obtain degrees of freedom (d.f.), mean squares (M.S.), and other intermediate statistics by PRESSING RCL _ _. (The contents of the memory registers are given below).

SAMPLE ANALYSIS OF VARIANCE TABLE

Source	d.f.	Expected Mean Square
Replicates	r-1	$\sigma^2_\varepsilon + b\sigma^2_\delta + ab\sigma^2_r$
Factor A	a-1	$\sigma^2_\varepsilon + b\sigma^2_\delta + rb/(a-1)\ \theta^2_a$
error a	(r-1)(a-1)	$\sigma^2_\varepsilon + b\sigma^2_\delta$
whole plot total	ar-1	
Factor B	b-1	$\sigma^2_\varepsilon + ra/(b-1)\ \theta^2_b$
Interaction A x B	(a-1)(b-1)	$\sigma^2_\varepsilon + r/(a-1)(b-1)\ \theta^2_{ab}$
error b	a(r-1)(b-1)	σ^2_ε
subplot total	ar(b-1)	
Total for analysis	abr-1	

STANDARD ERRORS OF THE MEAN ($s_{\bar{d}}$)

Comparison to be made	Formula
Means of Factor A averaged over all levels of Factor A	$\sqrt{(2)\ \text{EMS a}/rb}$
Means of Factor A at same level of Factor B	$\sqrt{2\{(b-1)\ \text{EMS b} + \text{EMS a}\}/rb}$
Means of Factor B averaged over all levels of Factor A	$\sqrt{(2)\ \text{EMS b}/ra}$
Means of Factor B at same level of Factor A	$\sqrt{(2)\ \text{EMS b}/r}$

Note: EMS = error mean square for the error term specified.

PROGRAM SEQUENCE FOR SPLIT PLOT ANALYSIS IN RANDOMIZED COMPLETE BLOCKS

FOR THE TEXAS INSTRUMENTS SR-52 CALCULATOR

CARD I

Location	Key					Location	Key					Location	Key				
000	LBL	A	STO	6	7	080	+	RCL	0	3	X^2	160	3	STO	0	4	STO
005	SUM	1	2	X^2	SUM	085	+	RCL	0	4	X^2	165	0	5	STO	0	6
010	1	4	1	SUM	1	090	+	RCL	0	5	X^2	170	STO	1	6	RCL	1
015	3	SUM	1	9	RCL	095	+	RCL	0	6	X^2	175	7	HLT	(	RCL	6
020	6	7	IND	SUM	1	100	)	SUM	0	8	RCL	180	1	X^2	+	RCL	6
025	3	HLT	LBL	B	RCL	105	1	6	X^2	SUM	0	185	2	X^2	+	RCL	6
030	1	2	SUM	1	6	110	9	RCL	0	1	SUM	190	3	X^2	+	RCL	6
035	SUM	1	1	X^2	SUM	115	6	1	RCL	0	2	195	4	X^2	+	RCL	6
040	0	7	RCL	1	2	120	SUM	6	2	RCL	0	200	5	X^2	+	RCL	6
045	÷	RCL	1	3	=	125	3	SUM	6	3	RCL	205	6	X^2	)	SUM	1
050	STO	6	7	1	SUM	130	0	4	SUM	6	4	210	0	HLT			
055	1	8	0	STO	1	135	RCL	0	5	SUM	6						
060	2	STO	1	3	RCL	140	5	RCL	0	6	SUM						
065	6	7	HLT	LBL	C	145	6	6	1	SUM	1						
070	(	RCL	0	1	X^2	150	7	0	STO	0	1						
075	+	RCL	0	2	X^2	155	STO	0	2	STO	0						

CONTENTS OF MEMORY REGISTERS

No.	Contents	No.	Contents
00	not used	14	ΣX^2
01	working total replicate 1	15	not used
02	working total replicate 2	16	working whole plots
03	working total replicate 3	17	whole plot counter
04	working total replicate 4	18	subplot counter
05	working total replicate 5	19	replicate counter
06	working total replicate 6	61	total replicate 1
07	Σ(subplot totals within whole plots)^2	62	total replicate 2
08	Σ(subplot totals within replicates)^2	63	total replicate 3
09	Σ(whole plot totals)^2	64	total replicate 4
10	Σ(replicate totals)^2	65	total replicate 5
11	ΣX	66	total replicate 6
12	working subplot total	67	X/subplot mean
13	working replicate counter		

CARD II

Location	Key					Location	Key					Location	Key				
000	LBL	A'	RCL	1	9	080	1	=	STO	0	2	160	÷	(	RCL	1	7
005	÷	RCL	1	8	=	085	RCL	0	8	÷	RCL	165	X	RCL	1	9	)
010	STO	1	9	RCL	1	090	1	8	-	RCL	0	170	-	RCL	0	1	=
015	8	÷	RCL	1	7	095	1	=	STO	0	3	175	STO	1	3	RCL	0
020	=	STO	I	8	HLT	100	RCL	I	0	÷	(	180	7	÷	RCL	1	9
025	LBL	A	X	RCL	1	105	RCL	1	7	X	RCL	185	-	RCL	0	1	-
030	9	=	SUM	1	5	110	1	8	)	-	RCL	190	RCL	0	9	-	RCL
035	HLT	LBL	B	RCL	1	115	0	1	=	STO	0	195	1	3	=	STO	0
040	5	X^2	SUM	1	6	120	4	RCL	0	9	÷	200	5	RCL	0	2	-
045	0	STO	1	5	HLT	125	(	RCL	1	8	X	205	RCL	0	3	-	RCL
050	LBL	C	RCL	1	1	130	RCL	1	9	)	-	210	1	3	-	RCL	0
055	X^2	÷	(	RCL	1	135	RCL	0	1	=	STO	215	5	=	STO	0	6
060	7	X	RCL	1	8	140	0	9	RCL	0	3	220	HLT				
065	X	RCL	1	9	)	145	-	RCL	0	4	-						
070	=	STO	0	1	RCL	150	RCL	0	9	=	STO						
075	1	4	-	RCL	0	155	1	2	RCL	1	6						

CONTENTS OF MEMORY REGISTERS

No.	Contents	No.	Contents
00	not used	10	Σ(replicate totals)^2 *
01	correction factor	11	ΣX *
02	total S.S.	12	error a S.S.
03	total S.S. for whole plots	13	Factor B S.S.
04	replicate S.S.	14	ΣX^2 *
05	Interaction A x B S.S.	15	working subplot totals
06	error b S.S.	16	Σ(subplot totals by levels of Factor B)^2
07	Σ(subplot totals within whole plots)^2 *	17	number of levels of Factor A
08	Σ(subplot totals within replicates)^2 *	18	number of levels of Factor B
09	Factor A S.S.	19	number of replicates

* - memory register transferred from previous Card

CARD III

Location	Key					Location	Key					Location	Key				
000	LBL	A'	RCL	1	9	080	:	RCL	0	8	=	160	÷	RCL	0	6	=
005	-	1	=	STO	0	085	STO	0	9	RCL	1	165	HLT	LBL	D	RCL	0
010	7	RCL	1	7	-	090	2	÷	RCL	1	0	170	5	÷	RCL	0	6
015	1	=	STO	0	8	095	=	STO	1	2	RCL	175	=	HLT	LBL	E	RCL
020	RCL	0	7	X	RCL	100	1	3	÷	RCL	1	180	1	1	÷	(	RCL
025	0	8	=	STO	1	105	4	=	STO	1	3	185	1	9	X	RCL	1
030	0	RCL	1	8	-	110	RCL	0	5	÷	RCL	190	8	X	RCL	1	7
035	1	=	STO	1	4	115	1	5	=	STO	0	195	)	=	STO	0	1
040	RCL	0	8	X	RCL	120	5	RCL	0	6	÷	200	RCL	1	2	√	÷
045	1	4	=	STO	1	125	RCL	1	6	=	STO	205	RCL	0	1	=	HLT
050	5	RCL	1	7	X	130	0	6	HLT	LBL	A	210	LBL	E'	RCL	0	6
055	RCL	0	7	X	RCL	135	RCL	0	4	÷	RCL	215	√	÷	RCL	0	1
060	1	4	=	STO	1	140	1	2	=	HLT	LBL	220	=	HLT			
065	6	RCL	0	4	÷	145	B	RCL	0	9	÷						
070	RCL	0	7	=	STO	150	RCL	1	2	=	HLT						
075	0	4	RCL	0	9	155	LBL	C	RCL	1	3						

CONTENTS OF MEMORY REGISTERS

No.	Contents	No.	Contents
00	not used	10	error a d.f.
01	grand mean X	11	X *
02	total S.S. *	12	error a M.S.
03	total S.S. for whole plots *	13	Factor B M.S.
04	replicates M.S.	14	Factor B d.f.
05	Interaction A x B M.S.	15	Interaction A x B d.f.
06	error b M.S.	16	error b d.f.
07	replicates d.f.	17	number of levels of Factor A *
08	Factor A d.f.	18	number of levels of Factor B *
09	Factor A M.S.	19	number of replicates *

* - memory register transferred from previous Card

APPENDIX

Numerical examples of the two-way and split plot analyses of variance in randomized complete blocks are given to help familiarize the reader with the instructions.

A. TWO-WAY ANALYSIS OF VARIANCE IN RANDOMIZED COMPLETE BLOCKS:

The program sequence and user instructions for the two-way analysis of variance may be found in Insecticide and Acaricide Tests 2:7-10. The following example illustrates the utility of the statistical tests for data analysis.

Three fungicides were tested for their efficacy against the fungus causing Southern stem rot in peanuts. Field plots, consisting of four, 50-foot rows, were arranged in randomized complete blocks in a field naturally infested with the fungus. Data consists of yields of the cultivar NC5 by plots.

Table 1. Plot yields of peanuts treated with fungicides (lb/plot)

Treatment	Replicate			
	1	2	3	4
A	29.9	30.9	27.7	28.8
B	22.6	27.1	26.1	23.4
C	15.2	17.2	20.6	20.2
Check	17.3	14.8	20.6	19.0

Table 2. Analysis of variance

Source	Degrees of freedom	Sums of squares	Mean squares	F value
Replications	3	12.87	4.29	0.86
Fungicides	3	361.64	120.55	24.20
Error	9	44.83	4.98	
Total	15	419.34		

LSD .05 = 3.57, Coefficient of variability = 9.9%.

Treatment Mean Comparisons

Two commonly used methods for testing treatment means for significant differences are the Least Significant Difference Test (LSD) and the Duncan's New Multiple Range Test. We recommend the use of the LSD because it has strong sensitivity for separating mean differences, it is widely adaptable to many experimental situations especially in cases where treatment comparisons are preplanned such as chemical rates or formulations, and it is easy to compute and report in publications. Duncan's New Multiple Range Test is more conservative in that it is more difficult to demonstrate significant differences among treatment means, and some researchers prefer its use to gain greater confidence in interpretation of the data. In experiments where large numbers of treatments are being compared, of which very little is known and no comparisons are pre-planned, the Duncan's Test is appropriate. This would be the case, for example, in a variety trial in which 30 or 40 cultivars are being screened for resistance to a new disease.

Least Significant Difference Test (LSD)

The LSD value for the two-way analysis of variance is machine-calculated and may be obtained directly as program output. Treatment means are declared significantly different if their difference is larger than the LSD value. In this example, Fungicides A and B are significantly different from each other and all other treatments. Fungicide C is not significantly different from the Check.

Duncan's New Multiple Range Test

The Duncan's multiple range groupings ('least significant ranges') are computed by entering the appropriate 'significant studentized ranges' from a statistical table. First rank the means being tested in ascending or decending order. In the example, if we choose to test Fungicide A versus the Check, there are four means in the multiple range grouping. From a statistical table, the 'significant studentized range' is 3.42 for a grouping of four means at 9 degrees of freedom (error d.f.) and the 5% level of protection. Enter this value in the calculator and PRESS: A, and 3.82 is displayed. The means of Fungicide A and Check differ by more than 3.82, therefore they are declared significantly different. Any two means may be tested simply by entering the appropriate 'significant studentized range'.

Data Presentation

The following table is a suggested format for the presentation of data analyzed by the two-way analysis of variance. Use LSD or DMR, not both; in this example the LSD test is preferred.

Table 1. Peanut yields in lb/plot

Treatment	lb/plot (means)*
Fungicide A	29.325 a
Fungicide B	24.800 b
Fungicide C	18.300 c
Check	17.925 c
LSD .05	3.57

*Means in a column followed by the same letter(s) are not significantly different (P = .05, DMR test).

B. SPLIT PLOT ANALYSIS OF VARIANCE IN RANDOMIZED COMPLETE BLOCKS:

Consider a test in which two varieties of tomato (Factor A or whole plots) and several nematicides (Factor B or subplots) are arranged in a split plot design in three randomized complete blocks. Plants are counted for the evidence of root knot nematodes, and data is given as numbers of infected plants per plot. Calculations of the different standard errors of the means and their application using the Least Significant Difference Test (LSD) for comparisons of the experimental means are given following the analysis of variance table.

Table 1. Numbers of infected plants per plot

Variety	Replication	Nematicides X	Y	Z
A	1	20	45	45
A	2	25	45	50
A	3	40	55	55
B	1	10	20	25
B	2	15	20	20
B	3	20	20	30

Table 2. Analysis of variance of the split plot

Source	Degree of freedom	Sums of squares	Mean squares	F value
Replications	2	286.11	146.03	5.42 **
Factor A (Varieties)	1	2222.22	2222.22	84.21 **
error a	2	52.78	26.39	
Factor B (Nematicides)	2	834.99	417.50	35.36 **
Interaction A x B	2	187.23	93.61	7.93 **
error b	8	94.44	11.81	
Total	17	3677.78		

Coefficient of Variability (whole plots) = 16.5%, Coefficient of Variability (subplots) = 11.0%.

Table 3. Standard errors of the mean

Difference between:	Standard error	Degrees of freedom for tabular 't'
Variety means	$\sqrt{\frac{(2)(26.39)}{(3)(3)}} = 2.42$	2
Variety means for same nematicide	$\sqrt{\frac{(2)[(2)(11.81) + 26.39]}{(3)(3)}} = 3.33$	-
Nematicide means	$\sqrt{\frac{(2)(11.81)}{(3)(2)}} = 1.98$	8
Nematicide means for same variety	$\sqrt{\frac{(2)(11.81)}{3}} = 2.81$	8

Least Significant Difference Test

LSD = t.05 (from statistical table at specified d.f.) x standard error of the mean

differences between variety means averaged over all nematicide treatments

t.05 = 4.303 for 2 d.f.

LSD .05 = (4.303)(2.42) = 10.41

--- any two variety means whose difference is larger than 10.41 are declared significantly different. Variety A is significantly different from Variety B.

Variety Means	
A	B
20.00	42.22

differences between variety means for same nematicide treatment

--- since this standard error of the mean is a weighted pool of the two error terms (error a and error b), the 't' value used in calculating the LSD must also be weighted.

$$t' .05 = \frac{(b-1)(\text{error b})(\text{'t'.05 for Factor B d.f.}) + (\text{error a})(\text{'t' for Factor A d.f.})}{(b-1)(\text{error b}) + (\text{error a})}$$

$$t' .05 = \frac{(2)(11.81)(2.306) + (26.39)(4.303)}{(2)(11.81) + (26.39)} = 3.360$$

LSD .05 = (3.360)(3.33) = 11.19

--- any two variety means whose difference is larger than 11.19 are declared significantly different. In this case, the two varieties are significantly different at each of the three nematicide treatments.

Treatment	Variety means A	Variety means B
for nematicide X	15.00	28.33
for nematicide Y	20.00	48.33
for nematicide Z	25.00	50.00

differences between nematicide means averaged over all varieties

t.05 = 2.306 for 8 d.f.

LSD .05 = (2.306)(1.98) = 4.57

--- any two nematicide means whose difference is larger than 4.57 are declared significantly different. Nematicide X is significantly different from nematicides Y and Z, but nematicide Y is not significantly different from nematicide Z.

Nematicide means X	Y	Z
21.67	34.17	37.50

differences between nematicides for same variety

t.05 = 2.306 for 8 d.f.

LSD .05 = (2.306)(2.81) = 6.48

--- any two nematicide means whose difference is larger than 6.48 are declared significantly different. For variety A, nematicide X is significantly different from nematicides Y and Z, but nematicide Y is not significantly different from nematicide Z. For variety B, none of the nematicide treatments are significantly different. The two varieties behave differently in response to nematicide treatments.

Variety	Nematicides means X	Y	Z
for variety A	28.33	48.33	50.00
for variety B	15.00	20.00	25.00

Duncan's New Multiple Range Tests

The calculations for the Duncan's Test are similar to, although more complex than those required for the LSD determination. Researchers who wish to use the Duncan's Test are referred to the book, Principles and Procedures of Statistics by Steele and Torrie, discussed earlier in the main text of this paper.

Data Presentation

The following table is a suggested format for the presentation of split plot data in Insecticide and Acaricide Tests.

Treatment	Number of infected plants per plot: Variety A	Variety B	Nematicide means
Nematicide X	28.33	15.00	21.67
Nematicide Y	48.33	20.00	34.17
Nematicide Z	50.00	25.00	37.50
Variety means	42.22	20.00	

Differences between variety means averaged over nematicides	LSD .05 = 10.41
Differences between variety means for same nematicide	LSD .05 = 11.19
Differences between nematicide means averaged over varieties	LSD .05 = 4.57
Differences between nematicide means for same variety	LSD .05 = 6.48