CHAPTER 1

The Standard Split Plot Experiment Design

1.1. INTRODUCTION

Prior to starting the topic of this book, it was deemed advisable to present some design concepts, definitions, and principles. Comparative experiments involve a number, v, treatments (factors) where a *treatment* is an item of interest to the experimenter. A treatment could be a medical treatment, a drug application, a level of a factor (amount of a drug, fertilizer, insecticide, etc.), a genotype, an agricultural practice, a marketing method, a teaching method, or any other item of interest. The selection of the v treatments for an experiment is known as the *treatment design*. The selection of an appropriate treatment design is a major element for the success of an experiment. It may include checks (standards, placebos) or other *points of reference*. The treatments may be all combinations of two or more factors and this is known as a *factorial arrangement* or *factorial treatment design*. A subset of a factorial is denoted as a *fractional replicate* of a factorial.

The arrangement of the treatments in an experiment is known as the *experiment design* or the *design of the experiment*. The term experimental design is of frequent use in statistical literature but is not used here. There are many types of experiment designs including: unblocked designs, blocked designs (complete blocks and incomplete blocks), row-column experiment designs, row-column designs within complete blocks, and others. Tables of designs are available in several statistical publications. However, many more experiment designs are available from a software package such as GENDEX (2005). This package obtains a randomized form of an experiment design and the design in variance optimal or near optimal.

There are three types of units to be considered when conducting an experiment. These are the observational unit, the sample or sampling unit, and the experimental

Variations on Split Plot and Split Block Experiment Designs, by Walter T. Federer and Freedom King

unit (Federer, 1991, Chapter 7). The *observational unit* is the smallest unit for which a response or measurement is obtained. A population or distribution is composed of *sample units or sampling units*. The *experimental unit* is the smallest amount of experimental material to which one treatment is applied. In many experiments, these three types of units are one and the same. In other cases, they may all be different. For example, suppose a treatment is a teaching method taught to a group of thirty students. The experimental unit is the group of thirty students for the period of time used to evaluate a teaching method. The sampling unit is the student, from a population of all students, for which inferences are to be made about this teaching method. Suppose that several examinations are given during the period of time the method is applied, the result from each examination is an observation or response and the observational unit is one examination from one student. In some investigations like sampling for water quality, obtaining a measurement on produce for a genotype from a plot of land measuring 1 m by 10 m (an experimental unit), etc., the sampling units are undefined.

Fisher (1966) presented three principles of experiment design. These are *local control (blocking, stratification), replication,* and *randomization*. Owing to random fluctuations of responses in any experiment or investigation, there is variation. The variation controlled should not be associated or interacting with treatment responses. For example, if an animal dies during the course of conducting an experiment and the death is not caused by the treatment, it should be considered as a missing observation and not as a zero response. Blocking (stratification) or local control is used to exclude extraneous variation in an experiment not associated with treatment effects. The blocking should be such as to have maximum variation among blocks and minimum variation within blocks. This makes for efficient experimentation and reduces the number of replicates (replications) needed for a specified degree of precision for treatment effects.

To reduce the effect of the variation in an experiment on measuring a treatment effect, the sample size or the number of replicates needs to be increased. Replication allows for an estimate of the random variation. Replication refers to the number of experimental units allocated to a particular treatment. The variation among the experimental units, eliminating treatment and blocking effects, is a measure of experimental variation or error. The number of replications should not be confused with the number of observations. For example, in a nutrition study of several regimes with an experimental unit consisting of one animal, weekly measurements (observations) may be taken on the weight of the animal over a 6-month period. These week-by-week measurements do not constitute replications. The number of replications is determined by the number of experimental units allocated to one treatment and not by the number of observations obtained.

Randomization is necessary in order to have a valid estimate of an error variance for comparing differences among treatments in an experiment. Fisher (1966) has defined a *valid estimate of an error variance or mean square* as one which contains all sources of variation affecting treatment effects except those due to the treatments themselves. This means that the estimated variance should be among experimental units treated alike and not necessarily among observations.

An appropriate response model needs to be determined for each experiment. It is essential to determine the pattern of variation in an experiment or investigation and not assume that one response model fits all experiments for a given design. With the availability of computers, *exploratory model selection* may be utilized to determine variation patterns in an experiment (Federer, 2003). The nature of the experiment design selected and the variation imposed during the conduct of an experiment determine the variation pattern. The conduct of an experiment or investigation is a part of the design of the experiment or investigation. This fact may be overlooked when selecting a response model equation for an experiment. For example, a randomized complete block design may be selected as the design of the experiment. Then, during the course of conducting the experiment, a part of the replicate of the experiment is flooded with water. This needs to be considered as a part of the design of the experiment and may be handled by setting up another block, using a covariate, or missing experimental units. This would not be the response model envisioned when the experiment design was selected. Or, it may be that the experimenter observed an unanticipated gradient in some or all of the blocks. A response model taking the gradients within blocks into consideration should be used in place of the model presumed to hold when the experiment was started. More detail on exploratory model selection may be found in Federer (2003).

For further discussion of the above, the reader is referred to Fisher (1966) and Federer (1984). The latter reference discusses a number of other principles and axioms to consider when conducting experiments.

An analysis of variance is considered to be a partitioning of the total variation into the variation for each of the sources of variation listed in a response model. An F-test is not considered to be a part of the analysis of variance as originally developed by Sir Ronald A. Fisher. Statistical publications often consider an F-test as part of the analysis of variance. We do not, as variance component estimation, multiple range tests, or other analyses may be used in connection with an analysis of variance. Some experimenters do consider the term analysis of variance to be a misnomer. A better term may be a partitioning of the total variation into its component parts or simply variation or variance partitioning.

1.2. STATISTICAL DESIGN

The standard split plot experiment design (SPED) discussed in several statistics textbooks has a two-factor factorial arrangement as the treatment design. One factor, say A with a levels, is designed as a randomized complete block design with r complete blocks or replicates. The experimental unit, the smallest unit to which one treatment is applied, for the levels of factor A treatments is called a *whole plot experimental unit* (wpeu). Then each wpeu is divided into b *split plot experimental units* (speus) for the b levels of the second factor, say B. Note that either or both factors A and B could be in a factorial arrangement or other treatment design rather than a single factor. A schematic layout of the standard SPED is shown below.

Standard split plot design with r replicates, a levels of factor A, and b levels of factor B

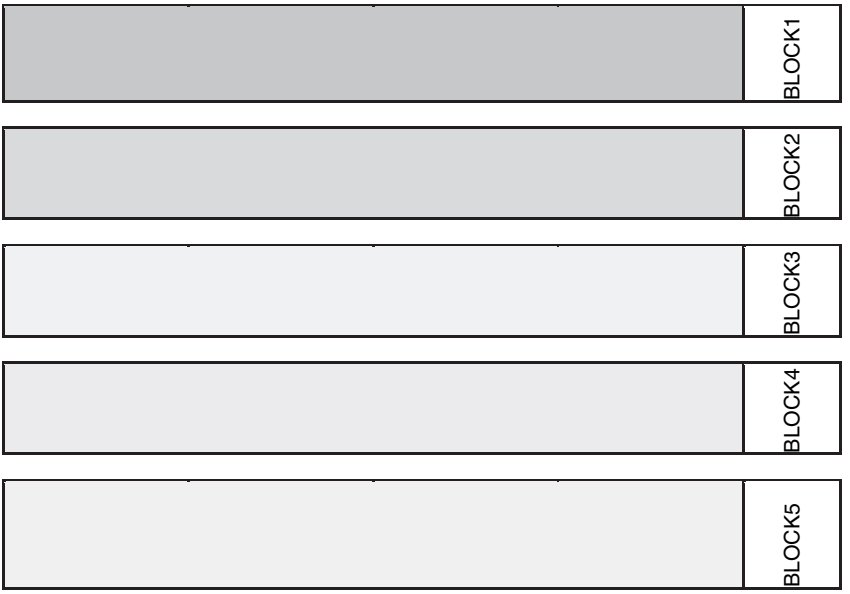
Replicate	1	2	3	...	r
Whole plot factor A	1 2 ... a	1 2 ... a	1 2 ... a	...	1 2 ... a
Split plot factor B	1 1 ... 1	1 1 ... 1	1 1 ... 1		1 1 ... 1
	2 2 ... 2	2 2 ... 2	2 2 ... 2		2 2 ... 2
	.....	.....	.....		.....
	b b...b	b b...b	b b...b		b b...b

The a levels of factor A are randomly and independently allocated to the a wpeus within *each* of the r complete blocks or replicates. Then within *each* wpeu, the b levels of factor B are independently randomized. There are r independent randomizations for the a levels of factor A and ra independently assigned randomizations for b levels of factor B. The fact that the *number of randomizations and the experimental units* are different for the two factors implies that each factor will have a separate error term for comparing effects of factor A and effects of factor B.

Even though the standard SPED has the whole plot factor A treatments in a randomized complete block design, any experiment design may be used for the factor A. For example, a completely randomized experiment design, a Latin square experiment design, an incomplete block experiment design, or any other experiment design may be used for the whole plot treatments. These variations are illustrated in Chapter 3.

The three steps in randomizing a plan for a standard or basic split plot experiment design consisting of $r = 5$ blocks (replicates), $a = 4$ levels of whole plot factor A, and $b = 8$ levels of split plot factor B are shown below:

Step 1: Divison of the experimental area or material into five blocks



Step 2: Randomizaton of four levels of whole plot factor *A* to each of five blocks

				BLOCK1
A3	A2	A1	A4	
				BLOCK2
A4	A1	A3	A2	
				BLOCK3
A2	A3	A4	A1	
				BLOCK4
A4	A2	A3	A1	
				BLOCK5
A3	A4	A1	A2	

Step 3: Randomization of eight levels of split plot factor B within each level of whole plot factor A

B1	B2	B7	B2	
B4	B3	B8	B4	
B5	B4	B4	B7	
B3	B5	B2	B5	
B6	B1	B5	B8	
B8	B6	B3	B1	
B7	B8	B6	B6	
B2	B7	B1	B3	BLOCK1
A3	A2	A1	A4	
B7	B6	B2	B5	
B2	B1	B3	B4	
B4	B4	B5	B2	
B6	B3	B7	B8	
B3	B7	B8	B3	
B8	B2	B1	B6	
B1	B5	B6	B7	
B5	B8	B4	B1	BLOCK2
A4	A1	A3	A2	
B4	B7	B1	B6	
B6	B8	B2	B1	
B1	B2	B4	B3	
B8	B6	B3	B5	
B5	B3	B7	B4	
B7	B1	B8	B8	
B3	B5	B6	B7	
B2	B4	B5	B2	BLOCK3
A2	A3	A4	A1	

B3	B7	B5	B8	
B8	B6	B2	B5	
B5	B2	B3	B4	
B6	B8	B4	B1	
B1	B4	B7	B3	
B7	B5	B6	B6	
B4	B1	B8	B7	
B2	B3	B1	B2	BLOCK4
A4	A2	A3	A1	
B3	B1	B7	B1	
B1	B6	B2	B7	
B5	B2	B3	B4	
B6	B7	B4	B8	
B8	B4	B5	B3	
B7	B5	B6	B6	
B4	B8	B1	B5	
B2	B3	B8	B2	BLOCK5
A3	A4	A1	A2	

If an experiment design involving blocking is used for the b split plot treatments, factor B, should be *within each* whole-plot-treatment wpeu, as this facilitates the statistical analysis for an experiment as orthogonality of effects is maintained. If the experiment design for the split plot factor B treatments is over levels of the whole plot treatments within one complete block, confounding of effects is introduced and the statistical analysis becomes more complex (Federer, 1975). This may not be a computational problem as available statistical software packages can be written to handle this situation. However, the confounding of effects reduces the precision of contrasts and estimates of effects.

1.3. EXAMPLES OF SPLIT-PLOT-DESIGNED EXPERIMENTS

Example 1—A seed germination test was conducted in a greenhouse on $a = 49$ genotypes of guayule, the whole plots (factor A), with four seed treatments (factor B) applied to each genotype as split plot treatments (Federer, 1946). The wpeu was a greenhouse flat for one genotype and 100 seeds of each of the four seed treatments (factor B) were planted in a flat, as more information on seed treatment than on genotype was desired and this fitted into the layout more easily than any other arrangement. The speu consisted of 1/4 of a greenhouse flat in which 100 seeds were planted. The 49 genotypes were arranged in a triple lattice incomplete block experiment design with $r = 6$ complete blocks and with an incomplete block size of $k = 7$ wpeus. The four seed treatments were randomly allocated to the four speus in a flat, that is, within each genotype wpeu. The data for eight of the 49 genotypes in three of the six replicates are given as Example X-1 of Federer (1955) and as Example 1.2. The whole plot treatments, 49 genotypes, are considered to be a random sample of genotypes from a population of genotypes, that is, they are

considered to be random effects whereas the seed treatments are fixed effects as these are the only ones of interest.

Example 2—Example X-2 of Federer (1955) contains the yield data for $b = 6$ genotypes which are corn double crosses. The data are from two of the twelve districts set up for testing corn hybrids in Iowa. The $a = 2$ districts are the whole plots, and the six corn double crosses, the split plot treatments, are arranged in a randomized complete block design within each district. The yield data (pounds of ear corn) arranged systematically are given below:

District 1, *A*					
Double-cross, factor *B*	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Total
1-1	34.6	33.4	36.5	33.0	137.5
2-2	34.5	39.1	35.4	35.6	144.6
4-3	30.1	30.8	35.0	33.3	129.2
15-45	31.3	29.3	29.7	33.2	123.5
8-38	32.8	35.7	36.0	34.0	138.5
7-39	30.7	35.5	35.3	30.6	132.1
Total	194.0	203.8	207.9	199.7	805.4

District 2, *A*					
Double-cross, factor *B*	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Total
1-1	33.1	24.6	33.8	34.6	126.1
2-2	46.4	36.9	36.3	45.3	164.9
4-3	32.3	38.7	37.5	37.6	146.1
15-43	37.5	39.2	39.1	34.1	149.9
8-38	31.2	40.8	46.1	44.1	162.2
7-39	35.8	38.2	38.8	39.6	152.4
Total	216.3	218.4	231.6	235.3	901.6

Example 3—Cochran and Cox (1957), page 300, present the data for an SPED with $a = 3$ recipes, the whole plots (factor A), for chocolate cakes baked at $b = 6$ temperatures, the split plots (factor B). The response was the breaking angle of the cake. Enough batter for one recipe was prepared for the six cakes to be baked at the six temperatures. That is, the wpeu was one batter for six cakes. The three recipes were arranged in a randomized complete block design with $r = 15$ replicates.

Example 4—Federer (1955), page 26 of the Problem Section, presents the data for an SPED with $a = 2$ whole plot treatments (factor A) of alfalfa or no alfalfa and $b = 5$ split plot treatments of bromegrass strains. The bromegrass strains were intercropped (mixed together) with the alfalfa and no alfalfa (See Federer,

1993, 1999). The whole plot treatments were arranged in a randomized complete block design with $r = 4$ replicates. The dry weights (grams) of hay arranged systematically are:

Bromegrass strain, factor B	Replicate 1 Factor A		Replicate 2 Factor A		Replicate 3 Factor A		Replicate 4 Factor A	
	alfalfa	alone	alfalfa	alone	alfalfa	alone	alfalfa	alone
a	730	786	1004	838	871	1033	844	867
b	601	1038	978	1111	1059	1380	1053	1229
c	840	1047	1099	1393	938	1208	1170	1433
d	844	993	990	970	965	1.308	1111	1311
e	768	883	1029	1130	909	1247	1124	1289

Example 5—Das and Giri (1979), page 150, present an example of three varieties forming the whole plots and $b = 4$ manurial treatments forming the split plots in an SPED with $r = 4$ replications.

Example 6—Gomez and Gomez (1984), page 102, give a numerical example of six levels of nitrogen applications forming the whole plots and $b = 4$ rice varieties forming the split plots in an SPED with $r = 3$ replications.

Example 7—Raghavarao (1983), page 255, presents a numerical example where the whole plots were $a = 3$ nitrogen levels and the $b = 4$ split plot treatments were insecticides in an SPED with $r = 4$ replications.

Example 8—Leonard and Clark (1938), Chapter 21, give a numerical example of a split plot experiment design with $a = 10$ maize hybrids as the whole plots of 36 hills (3 plants per hill). The wpeus were divided into thirds with 12 hills making up the speu. The $b = 3$ split plot treatments were seeds from the three generations F1, F2, and F3. Two replicates were used and the response was the yield of ear corn.

Example 9—In a setting other than agriculture, three types of schools (public, religious, and private) were the whole plots. Four types of teaching methods formed the split plots. This arrangement was replicated over r school districts. The response was the average score on standardized tests.

Example 10—Two types of shelters (barn and outdoor) were the whole plot factor A treatments and two types of shoes for horses were used as the factor B split plot treatments. There were to be $r = 5$ sets (replicates) of four horses used. Two horses, wpeu, of each set would be kept in a barn and two would be kept outdoors. One horse, speu, had one type of shoe and the second horse received the other type of shoe. The response was length of time required before reshoeing a horse was required.

Example 11—In a micro-array experiment, the two whole plot treatments were methods one and two. The two split plot treatments were red color-label 1 and green

color-label 2 for method 1 and were green color-label 1 and red color-label 2 for method 2. There were $r = 10$ sets of whole plots. The color by label interaction is completely confounded with method in the SPED experiment performed.

Example 12—Three types of managements (factor A) constituted the whole plots that consisted of a litter of six male rats. The $b = 6$ medical treatments (factor B) were the split plot treatments with one rat constituting the speu. Three litters, wpeus, were obtained from each of $r = 6$ laboratories.

Example 13—A randomized complete block experiment design with $a = 5$ treatments (factor A) and $r = 5$ replicates was conducted to determine the effect of the treatments on the yield and the quality of strawberries. The experiment was laid out in the field in five columns, the blocks or replicates, and five rows. Hence, this is a row-column design as far as spatial variation is concerned. A 5×5 Latin square experiment design should have been used but was not. The strawberries in each of the 25 wpeus were graded into $b = 4$ quality grades (factor B) that were the split plot treatments. Responses were the weight and the number of strawberries in each of the grades within a wpeu.

Example 14—Jarmasz et al. (2005) used several forms of a split plot experiment design to study human subject perceptions to various stimuli. The factor sex was not taken into account when analyzing the data presented in the paper. Taking the factor sex into account adds to the splitting of units and the complexity of the analysis. Several variations of the SPED were used. The split-plot-designed experiment is of frequent occurrence in this type of research investigation.

Numerous literature citations of split plot designs are given by Federer (1955) in the Problem Section at the end of the book. This type of design appears in many fields of inquiry and is of frequent occurrence. Kirk (1968) lists ten references as representative applications of split plot designs in literature involving learning and other psychological research. The Annual Reports of the Rothamsted Experiment Station, the International Rice Research Institute (IRRI), and other research organizations give data sets for split-plot-designed experiments.

1.4. ANALYSIS OF VARIANCE

A partitioning of the degrees of freedom in an analysis of variance table for the various sources of variation is one method for writing a linear model for a set of experimental data. Alternatively, writing a linear model in equation form is another way of presenting the sources of variation for an experiment. A linear response model for the SPED for fixed effects factors A and B is usually given as

$$Y_{hij} = \mu + \rho_h + \alpha_i + \delta_{hi} + \beta_j + \alpha\beta_{ij} + \varepsilon_{hij}, \tag{1.1}$$

where Y_{hij} is the response of the hijth speu,

μ is a general mean effect,

ρ_h is the hth replicate effect which is identically and independently distributed with mean zero and variance σ_ρ^2,

α_i is the effect of the ith whole plot factor A treatment,

δ_{hi} is a whole plot random error term which is identically and independently distributed with mean zero and variance σ_δ^2,

β_j is the effect of the jth split plot factor B treatment,

$\alpha\beta_{ij}$ is the interaction effect of the ith whole plot treatment with the jth split plot treatment, and

ε_{hij} is a split plot random error effect identically and independently distributed with mean zero and variance σ_ε^2.

The ρ_h, ε_{hi}, and δ_{hij} in Equation (1.1) are considered to be mutually independent variables.

Prior to calculating an analysis of variance, ANOVA table for the above response model, it is often instructive and enlightening to construct an ANOVA table for *each* whole plot as follows:

Whole plot level	A1		A2		...	Aa	
Source of variation	DF	SS	DF	SS	...	DF	SS
Total	rb	T1	rb	T2	...	rb	Ta
Correction for mean	1	C1	1	C2	...	1	Ca
Replicate	$r-1$	R1	$r-1$	R2	...	$r-1$	Ra
Split plot factor B	$b-1$	B1	$b-1$	B2	...	$b-1$	Ba
$R \times B$ = Error	$(r-1)(b-1)$	E1	$(r-1)(b-1)$	E2	...	$(r-1)(b-1)$	Ea

DF is degrees of freedom and SS is sum of squares. The dot notation is used which indicates that this is a sum over the subscripts replaced by a dot. The sums of squares for the ith whole plot treatment, $i = 1, 2, \ldots, a$, are:

$$\mathrm{Ti} = \sum_{h=1}^{r}\sum_{j=1}^{b} Y_{hij}^2$$

$$\mathrm{Ci} = \mathrm{Y}_{.i.}^2/\mathrm{br}$$

$$\mathrm{Ri} = \sum_{h=1}^{r} Y_{hi.}^2/b - Y_{.i.}^2/br = b\sum_{i=1}^{r}\left(\bar{y}_{hi.} - \bar{y}_{.i.}\right)^2$$

$$\mathrm{Bi} = \sum_{j=1}^{b} Y_{.ij}^2/r - Y_{.i.}^2/br = r\sum_{j=1}^{b}\left(\bar{y}_{.ij} - \bar{y}_{.i.}\right)^2.$$

These are the usual equations for computing sums of squares for data from a randomized complete block designed experiment. Ei is obtained by subtraction.

Data from a split-plot-designed experiment should not be analyzed as a three-factor factorial of the three factors A, B, and R. This is *not correct* as can be seen from the above and noting that the b $R \times B$ interactions are *nested* within whole plot treatments. This means that this interaction is completely confounded with the $R \times A \times B$ interaction. The replicates for different wpeus are not the same even though they may have the same numbering. They are from different parts of the experiment. The calculations can be performed but this does not validate the partition for these two interactions.

A combined ANOVA is easily obtained from the above analyses as indicated in the table that follows.

Source of variation	Degrees of freedom	Sum of squares
Total	rab	$\mathrm{T1} + \mathrm{T2} + \ldots + \mathrm{T}a$
Correction for mean	1	CFM Compute as usual
Whole plot treatment A	$a - 1$	$\mathrm{C1} + \mathrm{C2} + \ldots + \mathrm{C}a - \mathrm{CFM}$
Replicate within A	$a(r - 1)$	$\mathrm{R1} + \mathrm{R2} + \ldots + \mathrm{R}a$
Replicate	$r - 1$	Compute as usual
Error $A = R \times A$	$(a - 1)(r - 1)$	Subtraction
Split plot treatment B within A	$a(b - 1)$	$\mathrm{B1} + \mathrm{B2} + \ldots + \mathrm{B}a$
Split plot treatment B	$a - 1$	Compute as usual
$A \times B$	$(a - 1)(b - 1)$	Subtraction
Error $B = R \times B$ within A	$a(b - 1)(r - 1)$	$\mathrm{E1} + \mathrm{E2} + \ldots + \mathrm{E}a$

The Replicate within A sum of squares with $a(r - 1)$ degrees of freedom is the sum $\mathrm{R1} + \mathrm{R2} + \ldots + \mathrm{R}a$. This is the Replicate sum of squares + the Error A sum of squares. The additional sums of squares required for the above table are obtained from the following equations:

$$\mathrm{CFM} = Y_{\ldots}^2/abr$$

$$\text{Replicate} = \sum_{i=1}^{r} Y_{h..}^2/ab - Y_{\ldots}^2/abr$$

$$\text{Split plot treatment } B = \sum_{i=1}^{b} Y_{.j}^2/ar - Y_{\ldots}^2/abr.$$

Using this format for obtaining an ANOVA for an SPED can be enlightening for information on the nature of the factor B responses at each level of factor A and for observing the homogeneity of the error mean squares $\mathrm{Ei}/(rb - r - b - 1)$ at each level of factor A.

In the above form, it may be instructive in some situations to partition each of the Ei sum of squares into Tukey's one-degree-of-freedom for nonadditivity (see e.g., Snedecor and Cochran, 1980, Section 15.8) and a residual sum of squares with $rb - r - b$ degrees of freedom. Likewise, the $R \times A$ sum of squares may be partitioned

to check for nonadditivity. The formula for computing Tukey's one-degree-of-freedom sum of squares for a two-way layout is

$$\text{TNA} = \frac{\left[\sum_{h=1}^{r}\sum_{j=1}^{b} Y_{hij}(\bar{y}_{hi.} - \bar{y}_{.i.})(\bar{y}_{.ij} - \bar{y}_{.i.})\right]^2}{\sum_{h=1}^{r}(\bar{y}_{hi.} - \bar{y}_{.i.})^2 \sum_{j=1}^{b}(\bar{y}_{.ij} - \bar{y}_{.i.})^2}. \tag{1.2}$$

The mean of combination *hi* is $\bar{y}_{hi.}$. $\bar{y}_{.i.}$ is the *i*th whole plot mean. $\bar{y}_{..j}$ is the mean of the *j*th split plot treatment, and $\bar{y}_{.ij}$ is the mean of treatment combination *ij*. For the numerical example, Example 1.2, in Section 1.7 and $i = 0$, the differences of replicate means from the overall mean are $-5/12$, $-2/12$, and 7/12. The differences of seed treatment means from the overall mean are 500/12, $-156/12$, $-148/12$, and $-196/12$. The replicates by seed treatment responses for genotype 0 are:

	Seed treatment					
Replicate	0	1	2	3	Total	$\bar{y}_{h0.} - \bar{y}_{.0.}$
1	66	12	13	6	97	$-5/12$
2	63	10	13	12	98	$-2/12$
3	70	13	11	7	101	7/12
Total	199	35	37	25	296	—
$\bar{y}_{.0j} - \bar{y}_{.0.}$	500/12	$-156/12$	$-148/12$	$-196/12$		

Using Equation (1.2) for the above data, TNA is computed as:

$$\begin{aligned}&[66(500/12)(-5/12) + 63(500/12)(-2/12) + 70(500/12)(7/12)\\ &\quad + \ldots + 7(-196/12)(7/12)]^2/[\{(-5/12)^2 + \ldots + (-196/12)^2\}]\\ &\quad = [-1,145 + 65 + \ldots - 79 - 67]^2/(2.167/4)(6,972/3) = 2.80.\end{aligned}$$

1.5. F-TESTS

The replicate effects should always be considered as random effects. Considering them as fixed effects makes no sense as an experimenter is concerned with inferences beyond these particular replicates. This means that the Error *A* mean square is the appropriate error term for testing significance of whole plot treatment main effects, that is, factor *A* effects. Depending on the validity of the assumption that the Error *A* effects, δ_{hi}, are normally, identically, and independently distributed with zero mean and common variance σ_δ^2, that is, NIID(0,σ_δ^2), an F-test of the Factor *A* mean square divided by the Error *A* mean square is appropriate for testing the null hypothesis that the *A* effects are zero.

When the whole plot treatment effects are fixed effects and the assumption of normality of the random error effects is correct, an F-test of the null hypothesis of zero split plot treatment effects is performed using the Error B mean square. Likewise, an F-test to test the null hypothesis of zero $A \times B$ interaction effects is obtained using the Error B mean square. Note that the normality assumption is not crucial in most cases as an F-test is quite robust, especially when the number of degrees of freedom associated with the denominator mean square is not small.

When the whole plot treatments are random effects, the appropriate error mean square for testing the null hypothesis of zero split plot treatment effects is the $A \times B$ interaction mean square. The appropriate error term for testing the null hypothesis of zero $A \times B$ interaction effects is the Error B mean square.

When the split plot treatments are random effects and whole plot treatments are fixed effects, the appropriate error mean square for testing the null hypothesis for zero split plot treatment effects is the Error B mean square. For the interaction variance component for factors A and B defined as $\sigma^2_{\alpha\beta}$, the error mean square

$$\sigma^2_\varepsilon + b\sigma^2_\delta + \frac{ra\sigma^2_{\alpha\beta}}{a-1}$$

is the appropriate mean square for testing for zero factor A effects. The degrees of freedom associated with the above mean square are unknown and will need to be approximated (see, e.g., Snedecor and Cochran, 1980, Section 6.11). The expected value of the interaction mean square is

$$\sigma^2_\varepsilon + \frac{ar\sigma^2_{\alpha\beta}}{a-1}.$$

The following table presents the expected values of the mean squares in an analysis of variance table for factors A and B as fixed effects and as random effects:

Source of variation	Degrees of freedom	Expected value of mean square	
		Fixed A and B	Random A and B
Replicate	$r-1$	$\sigma^2_\varepsilon + b\sigma^2_\delta + ab\sigma^2_\rho$	$\sigma^2_\varepsilon + b\sigma^2_\delta + ab\sigma^2_\rho$
Factor A	$a-1$	$\sigma^2_\varepsilon + b\sigma^2_\delta + f(\alpha_i)$	$\sigma^2_\varepsilon + b\sigma^2_\delta + r\sigma^2_{\alpha\beta} + rb\sigma^2_\alpha$
Error A	$(a-1)(r-1)$	$\sigma^2_\varepsilon + b\sigma^2_\delta$	$\sigma^2_\varepsilon + b\sigma^2_\delta$
Factor B	$b-1$	$\sigma^2_\varepsilon + f(\beta_j)$	$\sigma^2_\varepsilon + r\sigma^2_{\alpha\beta} + ar\sigma^2_\beta$
$A \times B$	$(a-1)(b-1)$	$\sigma^2_\varepsilon + f(\alpha\beta_{ij})$	$\sigma^2_\varepsilon + r\sigma^2_{\alpha\beta}$
Error B	$a(b-1)(r-1)$	σ^2_ε	σ^2_ε

The variance components for factor A effects and factor B effects are σ^2_α *and* σ^2_β, respectively. The other variance components have been defined previously. The term $f(x)$ refers to a function of the sum of squares of the parameter x inside the parentheses.

A table showing the variance components in each of the mean squares for random replicate and random whole plot treatment effects and for fixed split plot treatment effects is given below:

Source of variation	Degrees of freedom	Expected value of mean square
Replicate	$r-1$	$\sigma_\varepsilon^2 + b\sigma_\delta^2 + ab\sigma_\rho^2$
Whole plot factor A	$a-1$	$\sigma_\varepsilon^2 + b\sigma_\delta^2 + rb\sigma_\alpha^2$
Error A	$(a-1)(r-1)$	$\sigma_\varepsilon^2 + b\sigma_\delta^2$
Split plot factor B	$b-1$	$\sigma_\varepsilon^2 + \dfrac{br\sigma_{\alpha\beta}^2}{b-1} + f(\beta_j)$
$A \times B$ interaction	$(a-1)(b-1)$	$\sigma_\varepsilon^2 + \dfrac{br\sigma_{\alpha\beta}^2}{b-1}$
Error B	$a(b-1)(r-1)$	σ_ε^2

For a given set of data, the term $f(\beta_j) = ar\sum_{i=1}^{b} \dfrac{\beta_j^2}{b-1}$ is a function of the factor B effects. When the factor A effects are fixed effects, the variance component $\sigma_{\alpha\beta}^2$ drops out of the factor B mean square.

To obtain the above expectations and to be consistent with assumptions for the fixed effects situation, note the following. The same assumptions for the random effects case cannot be those for the mixed effects and still be consistent with the fixed case. Furthermore, note that once a random level of a random factor, say A, has been selected, *all* of the interaction terms with factor B are present. There is not a population of interaction terms but only b of them. This accounts for the term $b/(b-1)$ in the expectation of the interaction mean square and in the factor B mean square.

1.6. STANDARD ERRORS FOR MEANS AND DIFFERENCES BETWEEN MEANS

The estimated standard error of difference between two factor A effects or means for fixed factor B effects is the square root of two times the Error A mean square divided by rb for $i \neq i'$, that is,

$$\mathrm{SE}(\bar{y}_{i.} - \bar{y}_{i'.}) = \sqrt{\frac{2 \text{ Error } A \text{ mean square}}{rb}}. \tag{1.3}$$

The estimated standard error of a difference between two factor B effects or means for fixed factor A effects is, $j \neq j'$,

$$\mathrm{SE}(\bar{y}_{.j} - \bar{y}_{.j'}) = \sqrt{\frac{2 \text{ Error } B \text{ mean square}}{ra}}. \tag{1.4}$$

The estimated standard error of a difference between two factor B effects or means for random factor A effects is

$$\mathrm{SE}(\bar{y}_{.j} - \bar{y}_{.j'}) = \sqrt{\frac{2\ A \times B \text{ interaction mean square}}{ra}}. \tag{1.5}$$

The estimated standard error of difference between two factor B effects or means at one level of factor A is

$$\mathrm{SE}(\bar{y}_{.ij} - \bar{y}_{.ij'}) = \sqrt{\frac{2 \text{ Error } B \text{ mean square}}{r}}. \tag{1.6}$$

This latter standard error of a difference may be seen from the ANOVAs presented for each whole plot treatment. The estimated standard error of a difference between two factor A effects or means at one level of factor B is

$$\mathrm{SE}(\bar{y}_{.ij} - \bar{y}_{.i'j}) = \sqrt{\frac{2[(b-1) \text{ Error } B + \text{Error } A]}{rb}}. \tag{1.7}$$

The degrees of freedom for the above standard error of a difference and the following are unknown and need to be approximated.

The standard error of a mean for a whole plot treatment with random replicate effects is

$$\mathrm{SE}(\bar{y}_{.i.}) = \sqrt{\frac{\text{Error } A + b\sigma_{\rho}^2}{rb}}. \tag{1.8}$$

The standard error of a mean for a split plot treatment with random replicate effects is

$$\mathrm{SE}(\bar{y}_{.j}) = \sqrt{\frac{\text{Error } B + a\,\sigma_{\rho}^2}{ra}}. \tag{1.9}$$

The standard error of an $A \times B$ interaction mean with random replicate effects is

$$\mathrm{SE}(\bar{y}_{.ij}) = \sqrt{\frac{\text{Error } B + \sigma_{\rho}^2 + \sigma_{\delta}^2}{r}}. \tag{1.10}$$

The estimated values for the above standard errors of a mean and for difference between two means, Equations (1.3)–(1.10), are obtained by substituting the numerical values for Error A, Error B, and the estimate of the pertinent variance component for the corresponding ones in the above equations. The estimated values are obtained from an analysis of the data from an experiment.

1.7. NUMERICAL EXAMPLES

Example 1.1. A maize yield trial was conducted to determine the effects of four methods, $a = 4$, of primary seedbed preparations (A1, A2, A3, and A4), factor A the whole plot treatments, and four methods, $b = 4$, of planting the corn kernels (B1, B2, B3, and B4), factor B the split plot treatments. The basic split plot experiment design contained $r = 4$ complete blocks or replicates. The four seedbed preparations were arranged in a random fashion within each of the four replicates. Then within each of the $4 \times 4 = 16$ seedbed preparations, the wpeu was divided into four areas or plots, speus, and the four methods of planting maize seeds were randomly assigned to the four speus. The object of this experiment was to compare seedbed preparations and planting methods. In addition, it was desirable to know if there was an interaction and whether it is necessary to use a particular planting method for each seedbed preparation. A systematized arrangement of the maize yields from the experiment in bushels per acre, are given in Table 1.1.

Table 1.1. Bushels per Acre Yield of Maize for Seedbed Preparations and Planting Methods.

	Planting methods				
Replicate	B1	B2	B3	B4	Total
		A1 = plowed at 7 inches			
1	82.8	46.2	78.6	77.7	285.3
2	72.2	51.6	70.9	73.6	268.3
3	72.9	53.6	69.8	70.3	266.6
4	74.6	57.0	69.6	72.3	273.5
Total	302.5	208.4	288.9	293.9	1093.7
		A2 = plowed at 4 inches			
1	74.1	49.1	72.0	66.1	261.3
2	76.2	53.8	71.8	65.5	267.3
3	71.1	43.7	67.6	66.2	248.6
4	67.8	58.8	60.6	60.6	247.8
Total	289.2	205.4	272.0	258.4	1025.0
		A3 = blank basin listed			
1	68.4	54.5	72.0	70.6	265.5
2	68.2	47.6	76.7	75.4	267.9
3	67.1	46.4	70.7	66.2	250.4
4	65.6	53.3	65.6	69.2	253.7
Total	269.3	201.8	285.0	281.4	1037.5
		A4 = disk-harrowed			
1	71.5	50.9	76.4	75.1	273.9
2	70.4	65.0	75.8	75.8	287.0
3	72.5	54.9	67.6	75.2	270.2
4	67.8	50.2	65.6	63.3	246.9
Total	282.2	221.0	285.4	289.4	1078.0
B total	1142.2	836.6	1131.3	1123.1	

Table 1.2. Analysis of Variance and F-Statistics for the Data of Table 1.1.

Source of variation	DF	Sum of squares	Mean square	F	Prob>F
Total	64	285,505.47			
Correction for mean	1	279,991.26			
Replicate = R	3	223.81	74.60		
Factor A	3	194.56	64.85	3.69	0.06
A1 + A4 vs. A2 + A3	1	186.32		10.60	0.01
Rest	2	8.24		0.47	
Error $A = R \times A$	9	158.24	17.58		
Planting method = B	3	4107.38	1369.13	81.01	0.00
B2 vs. rest	1	4105.15		242.90	0.00
Rest	2	2.23		0.13	
$A \times B$ interaction	9	221.74	24.64	1.46	0.20
Error B	36	608.48	16.90		

The grand total is 4234.2. The replicate totals are 1086.0, 1090.5, 1035.8, and 1021.9 for replicates 1, 2, 3, and 4, respectively. An analysis of variance and F-statistics for this experiment are given in Table 1.2. An SAS computer program for computing this analysis of variance table is given in Appendix 1.1.

The sum of squares for the contrast A1 + A4 − A2 − A3 is computed as

$$\frac{(1093.7 + 1078.0 - 1025.0 - 1037.5)^2}{64} = 186.32.$$

The sum of squares for the contrast 3(B2) − B1 − B3 − B4 is computed as

$$\frac{[3(836.6) - 1143.2 - 1131.3 - 1123.1]^2}{16(3^2 + 1 + 1 + 1)} = \frac{(-887.8)^2}{192} = 4105.15.$$

As may be observed, these two contrasts account for most of the differences among the planting methods and seedbed preparations. There is a slight indication that some interaction may be present. Also, since the two "Rest" mean squares are less than the Error A and Error B mean squares, there appears to be some type of heterogeneity that is not controlled. The problem of finding it is left as an exercise for the reader as is the computation for the interaction of the above two contrasts. Figures 1.1 and 1.2 illustrate the variation of planting methods in each of the seedbed preparations with two different axes.

A computer code for obtaining many of the numerical results including the means is given in Appendix 1.1.

Example 1.2. An experiment consisting of 49 guayule genotypes as whole plot treatments was designed as a triple lattice incomplete block experiment design with $r = 6$ replicates (see Federer, 1946). The split plot treatment represented four seed treatments for breaking the dormancy of guayule seeds. The split plot experimental unit consisted of 100 seeds planted in one-fourth of greenhouse flat. The wpeu was a greenhouse flat. Eight of the guayule genotypes from three of the six replicates from

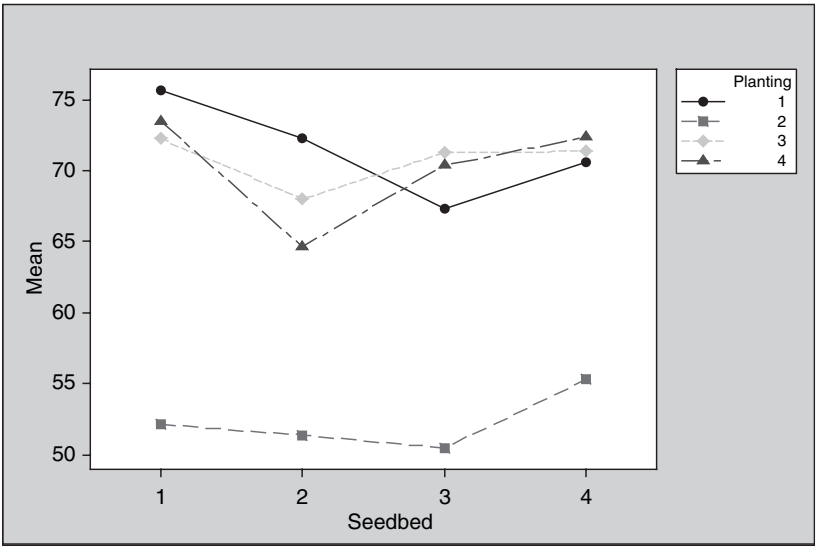


Figure 1.1. Planting method by seedbed preparation interaction.

this experiment were selected to illustrate the analysis for a split-plot-designed example. The selected data are analyzed as if a randomized complete block design had been used for the eight whole plot treatments. This design is now considered to be a standard split-plot-designed experiment. The data for the *ij* combinations of

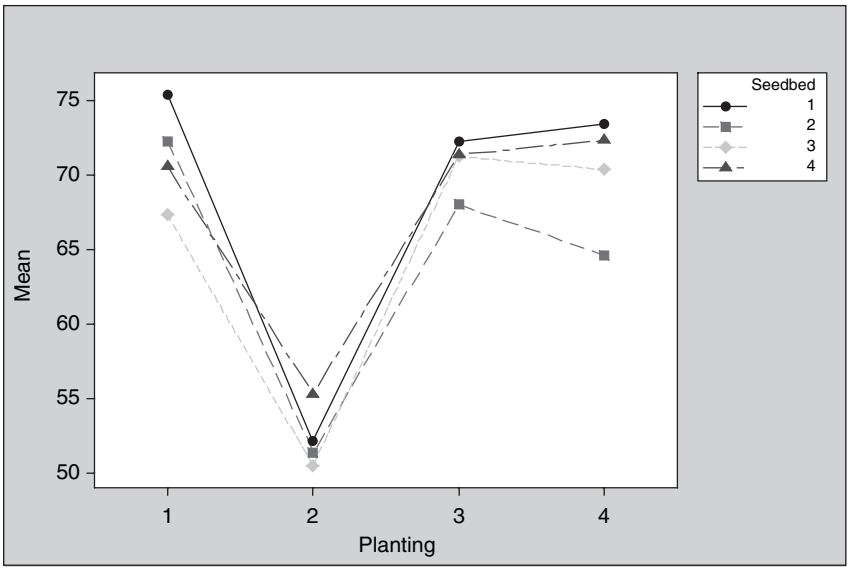


Figure 1.2. Planting method by seedling preparation interaction.

genotypes $i = 0, 1, \ldots, 7$ and seed treatments $j = 0, 1, 2, 3$, for each of the replicates $h = 1, 2, 3$ are given in Table 1.3. The top number of a pair is the combination ij and the bottom number is the number of plants that emerged from 100 seeds. A computer program for computing the analysis of variance tables and means is given in Appendix 1.2. The genotype-by-seed treatment totals are given in Table 1.4. The ANOVAs for seed treatments by replicate for each genotype are presented in Table 1.5. An ANOVA for this split-plot-designed example is given in Table 1.6.

Table 1.3. Number of Plants Germinating from 100 Seeds for Each of Four Seed Treatments from Eight Guayule Genotypes in a Split Plot Experiment Design with Three Replicates.

	Replicate 1								
	01	23	30	52	42	11	73	61	
	12	10	52	28	9	26	9	12	
	02	20	33	53	43	12	71	62	
	13	51	13	14	12	27	14	26	
	00	21	32	51	40	10	72	63	
	66	8	19	8	45	77	30	15	
	03	22	31	50	41	13	70	60	
	6	20	4	59	20	15	49	56	
Total	97	89	88	109	86	145	102	109	825
	Replicate 2								
	32	60	73	41	03	12	51	21	
	16	38	15	13	12	5	8	16	
	31	62	70	43	00	10	52	22	
	15	16	41	12	63	47	32	30	
	33	61	72	40	02	11	53	20	
	9	16	28	51	13	11	21	81	
	30	63	71	42	01	13	50	23	
	40	8	20	10	10	4	66	14	
Total	80	78	104	86	98	67	127	141	781
	Replicate 3								
	63	72	50	42	32	22	01	11	
	7	36	49	12	7	29	13	18	
	62	71	52	40	30	21	03	10	
	24	25	29	52	59	14	7	66	
	61	70	53	41	31	23	00	12	
	16	54	16	16	11	10	70	11	
	00	73	51	43	33	20	02	13	
	45	12	8	11	7	63	11	15	
Total	92	127	102	91	84	116	101	110	823

Table 1.4. Genotype-by-Seed Treatment Totals.

	Genotypes								
Seed treatment	0	1	2	3	4	5	6	7	Total
0	199	190	195	151	148	174	139	144	1340
1	35	55	38	30	49	24	44	59	334
2	37	43	79	42	31	89	66	54	481
3	25	34	34	29	35	51	30	36	274
Genotype total	296	322	346	252	263	338	279	333	2449

From Table 1.5, we note that the residual mean squares vary from 8.417 for genotype 0 to 55.889 for genotype 3. This may indicate that a square root or arcsine transformation of the numbers is needed. This was not done as differences in seed treatment means are large and a more precise analysis may not be necessary. The F-values for seed treatments varied from 20.74 for genotype 3 to 276.12 for

Table 1.5. ANOVAs and F-Values for Replicate and Seed Treatment for Each Genotype, SS = Sum of squares, MS = Mean Square.

Genotype 0

Source	DF	SS	MS	F-value	Prob > F
Total	12	14,325.98	—	—	—
Correction for mean	1	7301.31	—	—	—
Replicate	2	2.167	1.08	0.13	0.88
Seed treatment	3	6972.00	2324.00	276.12	0.00
Residual	6	50.50	8.42	—	—

Genotype 1

Source	DF	SS	MS	F-value	Prob > F
Total	12	14,955.99	—	—	—
Correction for mean	1	8640.32	—	—	—
Replicate	2	763.17	381.58	15.31	0.00
Seed treatment	3	5403.00	1801.00	72.28	0.00
Residual	6	149.50	24.92	—	—

Genotype 2

Source	DF	SS	MS	F-value	Prob > F
Total	12	16,184.00	—	—	—
Correction for mean	1	9976.33	—	—	—
Replicate	2	338.17	169.08	4.53	0.06
Seed treatment	3	5645.67	1881.89	50.45	0.00
Residual	6	223.83	37.31	—	—

Table 1.5. (*Continued*)

Genotype 3

Source	DF	SS	MS	F-value	Prob > F
Total	12	9112.00	—	—	—
Correction for mean	1	5292.00	—	—	—
Replicate	2	8.000	4.00	0.07	0.93
Seed treatment	3	3476.67	1158.89	20.74	0.00
Residual	6	335.33	55.89	—	—

Genotype 4

Source	DF	SS	MS	F-value	Prob > F
Total	12	8889.00	—	—	—
Correction for mean	1	5764.09	—	—	—
Replicate	2	4.17	2.08	0.23	0.80
Seed treatment	3	3066.25	1022.08	112.52	0.00
Residual	6	54.50	9.08	—	—

Genotype 5

Source	DF	SS	MS	F-value	Prob > F
Total	12	13,972.00	—	—	—
Correction for mean	1	9520.34	—	—	—
Replicate	2	83.167	41.58	2.56	0.16
Seed treatment	3	4271.00	1423.67	87.61	0.00
Residual	6	97.50	16.25	—	—

Genotype 6

Source	DF	SS	MS	F-value	Prob > F
Total	12	9107.00	—	—	—
Correction for mean	1	6486.75	—	—	—
Replicate	2	120.50	60.25	2.43	0.17
Seed treatment	3	2350.92	783.64	31.59	0.00
Residual	6	148.83	24.81	—	—

Genotype 7

Source	DF	SS	MS	F-value	Prob > F
Total	12	11,649	—	—	—
Correction for mean	1	9240.75	—	—	—
Replicate	2	96.50	48.25	2.82	0.14
Seed treatment	3	2208.92	736.31	42.96	0.00
Residual	6	102.83	17.14	—	—

Table 1.6. An Analysis of Variance and F-Values for the Data of Table 1.3.

Source of variation	DF	Sum of squares	Mean square	F-value	Prob > F
Total	96	98195	—	—	—
Correction for mean	1	61,458.76	—	—	—
Replicate	2	38.58	19.29	0.20	—
Genotype = *A*	7	763.16	109.02	1.11	—
Error *A*	14	1377.25	98.38	—	
Seed treatment = *B*	3	30,774.28	10,258.09	82.22	0.00
0 vs. 1 + 2 + 3	1	29,829.03		239.07	0.00
1 vs. 2 + 3	1	52.56		0.42	
2 vs. 3	1	892.69		7.15	0.02
Seed treatment × genotype	21	2620.13	124.77	5.15	0.00
A × 0 vs 1 + 2 + 3	7	1456.72	208.10	8.59	0.00
A × 1 vs. 2 + 3	7	632.94	90.42	3.73	0.01
A × 2 vs. 3	7	530.48	75.78	3.13	0.01
Error *B*	48	1162.84	24.23	—	—

genotype 0. There was smaller variation among replicate means, as the F-values varied from 0.07 for genotype 3 to 4.53 for genotype 2.

From the combined analysis in Table 1.6, it is noted that the seed treatment by genotype interaction is present. To study this in further detail, Figure 1.3 was prepared. Genotypes 0, 1, 2, and 5 for seed treatment 0 and genotypes 2 and 5 for

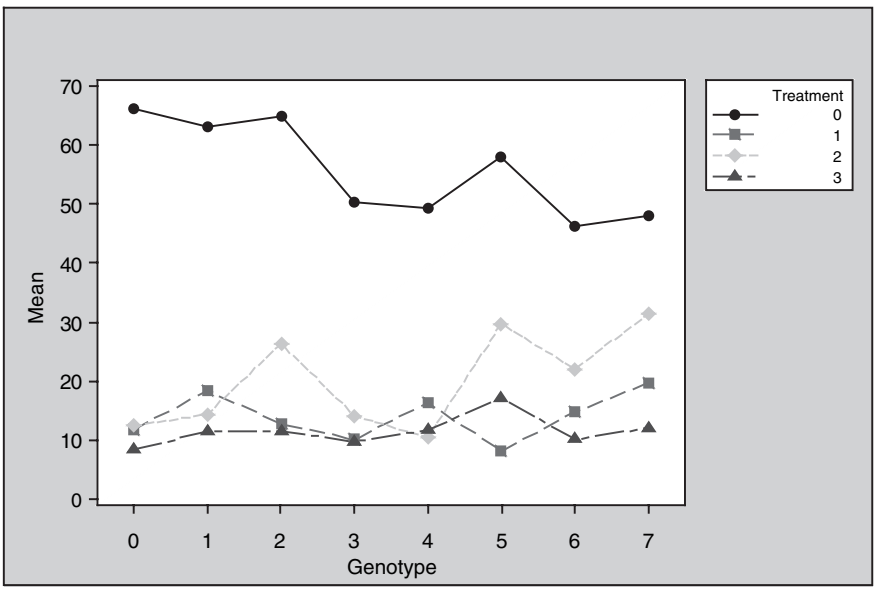


Figure 1.3. Genotype by seed treatment interaction.

seed treatment 2 are the ones contributing most to the interaction sum of squares. Their responses to seed treatments 0 and 2 were relatively higher than the other responses.

The various standard errors of a difference of two means are obtained next. The standard error of a difference between two genotype means is [Equation (1.3)].

$$\mathrm{SE}(\bar{y}_{.i.} - \bar{y}_{.i'.}) = \sqrt{\frac{2(98.38)}{3(4)}} = \sqrt{16.3967} = 4.05.$$

The standard error of a difference of two seed treatment means for random genotype effects is [Equation (1.4)].

$$\mathrm{SE}(\bar{y}_{..j} - \bar{y}_{..j'}) = \sqrt{\frac{2(124.77)}{3(8)}} = \sqrt{10.3975} = 3.22.$$

The standard error of difference between two seed treatment means for one genotype is [Equation (1.6)].

$$\mathrm{SE}(\bar{y}_{.ij} - \bar{y}_{.ij'}) = \sqrt{\frac{2(24.23)}{3}} = 4.02.$$

The standard error of difference between two genotype means for one seed treatment is [Equation (1.7)].

$$\mathrm{SE}(\bar{y}_{.ij} - \bar{y}_{.i'j}) = \sqrt{\frac{2\{3(24.23) + 98.38\}}{3(4)}} = \sqrt{28.5117} = 5.34.$$

Even though a seed treatment by genotype interaction is present (see Figure 1.3.), the mean for seed treatment 0, threshed seed and untreated, is far above the remaining three seed treatment means in increasing the germination percentage of guayule seeds, hence would be preferred for treating guayule seeds. Seed treatment 1 was unthreshed and untreated, seed treatment 2 was unthreshed and treated 1943 seeds, and seed treatment 3 was unthreshed and treated 1942 seeds. The seed treatment 3 represented the seed treatment method prior to this experiment. Seed treatment 0 involved using threshed and untreated seeds and was quite effective in increasing seed germination. The threshing removed many empty seeds. There are also genetic differences in a genotype response to seed treatment 0 but in all cases this treatment was superior to the other treatments. Some genotypes may have more empty seeds than others. A further experiment using different lengths of threshing times or other treatments may be needed when specific genotypes are being considered.

1.8. MULTIPLE COMPARISONS OF MEANS

Federer and McCulloch (1984) presented multiple comparison procedures for an SPED. They discussed five different multiple comparison procedures, viz., the least

significant difference procedure (lsd) with a per comparison error rate, Tukey's range procedure or honestly significant difference (hsd) with an experiment-wise error rate for all pairs of two means, the Bonferroni procedure (esd) with a per experiment error rate for m specified contrasts, Scheffe's procedure (ssd) with an error rate for all possible comparisons and contrasts, and Dunnett's procedure (dsd) for comparing treatments with a control with an experiment-wise error rate.

Using the data given in Table 1.3, all possible differences between pairs of the eight guayule genotype (whole plot) means are given in Table 1.7.

All possible differences between pairs of the seed treatment (split plot) means are presented in Table 1.8.

A two-sided Type I error rate of 5% is used. For genotype mean differences, the lsd is computed as (df = degrees of freedom associated with error term and E_a is the Error A mean square),

$$t_{.05,14\text{df}}\sqrt{\frac{2E_a}{rb}} = 2.145\sqrt{\frac{2(98.38)}{3(4)}} = 8.69. \tag{1.11}$$

Using Tukey's procedure, the hsd is computed as,

$$q_{.05,8,14\text{df}}\sqrt{\frac{E_a}{rb}} = 4.99\sqrt{\frac{98.38}{3(4)}} = 14.29. \tag{1.12}$$

Using the Bonferroni procedure, the esd, for $m = \dfrac{8(8-1)}{2} = 28$, is computed as,

$$\text{esd} = t_{.05/m,14\text{df}}\sqrt{\frac{2E_a}{rb}} = 3.85\sqrt{\frac{2(98.38)}{3(4)}} = 15.57. \tag{1.13}$$

Table 1.7. All Possible Differences Between Pairs of Eight Guayule Genotype Means, Number of Seeds Germinated Out of 100.

		Genotype						
		2	5	7	1	0	6	4
Genotype	Mean	29	28	28	27	25	23	22
3	21	8	7	7	6	4	2	1
4	22	7	6	6	5	3	1	—
6	23	6	5	5	4	2	—	—
0	25	4	3	3	2	—	—	—
1	27	2	1	1	—	—	—	—
7	28	1	0	—	—	—	—	—
5	28	1	—	—	—	—	—	—

Table 1.8. All Possible Differences Between Pairs of the four Seed Treatment Means, Number of Seeds Germinated out of 100.

		Seed treatment		
		0	2	1
Seed treatment	Mean	56	20	14
3	11	45	9	3
1	14	42	6	—
2	20	36	—	—

Using Scheffe's method, the ssd is computed as (df = degrees of freedom for Error A),

$$\text{ssd} = \sqrt{\frac{2(a-1)F_{.05}(a-1,\text{df})(E_a)}{rb}} = \sqrt{7(2.77)(2)\left\{\frac{98.38}{3(4)}\right\}} = 17.83. \quad (1.14)$$

Using the Dunnett method, the dsd is computed with genotype 2, for example, designated as the control treatment,

$$\text{dsd} = d_{a-1,\text{df},.05}\sqrt{\frac{2E_a}{rb}} = 3.10(4.045) = 12.54. \quad (1.15)$$

To compare differences between seed treatment means for random genotype effects, the lsd is computed as (E_{ab} is the interaction mean square),

$$\text{lsd} = t_{.05,21\text{df}}\sqrt{\frac{2E_{ab}}{ra}} = 2.08\sqrt{\frac{2(124.77)}{3(8)}} = 6.71. \quad (1.16)$$

The hsd is computed as,

$$q_{.05,4,21}\sqrt{\frac{E_{ab}}{ra}} = 3.96\sqrt{\frac{124.77}{3(8)}} = 9.03. \quad (1.17)$$

The esd, for $m = 4(4-1)/2 = 6$, is computed as,

$$\text{esd} = t_{.05/m,21\text{df}}\sqrt{\frac{2(E_{ab})}{ra}} = 3.82\sqrt{\frac{2(124.77)}{3(8)}} = 9.13. \quad (1.18)$$

The ssd is computed as (df = degrees of freedom for error mean square for seed treatments),

$$\text{ssd} = \sqrt{\frac{2(b-1)F_{.05}(b-1,\text{df})(E_{ab})}{ra}} = \sqrt{3(3.07)(2)\left\{\frac{124.77}{3(8)}\right\}} = 9.79. \quad (1.19)$$

The dsd is computed as, where seed treatment 2 is designated as the control treatment,

$$\text{dsd} = d_{b-1,\text{df},.05}\sqrt{\frac{2(E_{ab})}{rb}} = 2.56\sqrt{\frac{2(124.77)}{3(8)}} = 2.56(3.225) = 8.25. \quad (1.20)$$

In order to obtain the values for hsd, esd, and dsd for large values, say $ab = 32$, more extensive tables of t, q, and d values are needed. For the 32 genotype by seed treatment mean differences, the lsd for comparing seed treatment means for a specific genotype is computed as

$$\text{lsd} = t_{.05,48\text{df}}\sqrt{\frac{2E_b}{r}} = 2.01\sqrt{\frac{2(24.23)}{3}} = 2.01(4.019) = 8.08. \quad (1.21)$$

The ssd is computed as,

$$\text{ssd} = \sqrt{\frac{2(ab-1)F_{.05}(ab-1,\text{df})(E_b)}{r}} = \sqrt{2(31)(1.76)\left\{\frac{24.23}{3}\right\}} = 29.69. \quad (1.22)$$

The above multiple comparison results were for the genotype and seed treatment means. If it is desirable to perform multiple comparisons on the 32 genotypes by seed treatment means, the standard error of means and of differences between two means given in Equations (1.3)–(1.10) will need to be used. Depending upon which pair of means and method is under consideration, the appropriate standard error of a mean or of a difference between two means will need to be selected for each pair.

1.9. ONE REPLICATE OF A SPLIT PLOT EXPERIMENT DESIGN AND MISSING OBSERVATIONS

In the course of statistical consulting, many types of experiment designs are encountered. Federer (1975) presents an example wherein only one replicate of a split plot experiment design was used, and the experimenter wanted advice on the statistical analysis. Three light intensities were the whole plot treatments and were used in three different growth chambers. The three plant types were the split plot treatments. The plant types were tomato, pigweed, and pigweed + tomato (an intercropping treatment as described by Federer, 1993, 1999). One greenhouse flat of

eight plants formed the speu. Eight tomato plants and eight pigweed plants were used for the tomato and pigweed plant types. Four pigweed and four tomato plants made up the tomato + pigweed treatment. An ANOVA for one light intensity in one growth chamber is as given below in the tabular form.

Source of variation	DF	DF general
Total	24	bk
Correction for mean	1	1
Replicates (blocks) $= R$	0	0
Plant types $= B$	2	$b-1$
$R \times B$	0	0
Plants within B	21	$b(k-1)$

The $R \times B$ error mean square is the appropriate error term for comparing plant-type means but there is no estimate of it. The plants within B mean square are frequently used in place of the $R \times B$ interaction mean square. This is incorrect in that the variance component due to variation from block to block is not included in the plants within B mean square but it is in the plant-type mean square. This is a frequent mistake found in published literature. An ANOVA partitioning of the degrees of freedom for all 72 observations is as given below in the tabular form.

Source of variation	DF	DF general
Total	72	abk
Correction for mean	1	1
Replicates (blocks) $= R$	0	0
Light intensity = chamber $= A$	2	$a-1$
Error $A = R \times A$	0	0
Plant types $= B$	2	$b-1$
$A \times B$	4	$(a-1)(b-1)$
Error $B = R \times B$ within A	0	0
Plants within R, A, and B	63	$ab(k-1)$

If light intensity could be considered to be a random effect, then the $A \times B$ mean square would be used as the error term for comparing the plant-type means but it has only four degrees of freedom. The fact that there are $63 = ab(k-1)$ degrees of freedom associated with the plants within R, A, and B mean squares, has tempted many researchers to use these mean squares to replace the appropriate Error B mean square. This practice results in using an error mean square that is too small, resulting in false significance statements.

Occasionally missing observations occur in split-plot-designed experiments. Anderson (1946) and Khargonkar (1948) present formulas for computing missing plot values. Computer packages are mostly designed to handle these situations and hence there is no need for the formulas.

1.10. NATURE OF EXPERIMENTAL VARIATION

In Section 1.5, several assumptions about the nature of the random error terms were made to obtain the expected values of the various mean squares. The split plot random errors ε_{hij} were assumed to be identically and independently distributed, IID(0,σ_ε^2). It was further assumed that the Error A mean square contained both terms σ_ε^2 and σ_δ^2 with the split plot, whole plot random errors being additives. Also, it was assumed that the split plot and whole plot random error terms were independent. Simply because this was assumed and because it appears to be a reasonable assumption, does not mean that the assumption holds for all split-plot-designed experiments.

Cochran and Cox (1957, Section 7.12) and Kirk (1968, Chapter 8) present another way of quantifying the experimental variation exhibited by a split-plot-designed experiment. These authors assume that the split plot random errors ε_{hij} are correlated. For spatially laid out experiments, this correlation may be due to the proximity of neighboring speus. In baking and industrial experiments, a single batch may be divided into b speus for the b split plot treatments. Any factor affecting the batch affects all b speus. They assumed that the following correlation structure holds:

$$E[\varepsilon_{hik}\varepsilon_{hij}] = \rho\sigma_\varepsilon^2 \text{ and } E[\varepsilon_{hij}\varepsilon_{rst}] = 0,\ j \neq k,\ hij \neq rst \tag{1.23}$$

The random split plot error terms in the same whole plot have covariance $\rho\sigma_\varepsilon^2$ and those in different whole plots have zero covariance, that is, they are un-correlated.

Consider the case where factor B has $b = 2$ levels. Ignoring the random nature of the replicate or complete block effect, the variance of a whole plot is

$$E[(\varepsilon_{hi1} + \varepsilon_{hi2})^2] = \sigma_\varepsilon^2 + \sigma_\varepsilon^2 + 2\rho\sigma_\varepsilon^2 = 2\sigma_\varepsilon^2(1 + \rho). \tag{1.24}$$

For b levels of split plot treatments, the whole plot variance is,

$$E[(\varepsilon_{\text{hi}1} + \varepsilon_{\text{hi}2} + \varepsilon_{\text{hi}3} + \cdots + \varepsilon_{\text{hi}b})^2] = b\sigma_\varepsilon^2(1 + (b-1)\rho). \tag{1.25}$$

The split plot main effects are derived from differences of split plot responses. Hence the variance of a difference of two split plot treatments is,

$$E[(\varepsilon_{\text{hi}1} - \varepsilon_{\text{hi}2})^2] = \sigma_\varepsilon^2 + \sigma_\varepsilon^2 - 2\rho\sigma_\varepsilon^2 = 2\sigma_\varepsilon^2(1 - \rho), \tag{1.26}$$

with an effective error variance per speu of $\sigma_\varepsilon^2(1 - \rho)$ whatever the value of b. This variance also applies to contrasts of interaction effects within the same whole plot. For comparing two interaction terms from different whole plots the variance is $2\sigma_\varepsilon^2$. The analysis of variance, as described above, gives unbiased and correct estimates of the above.

For many situations ρ will be positive. However, it could be negative for certain types of experimental variations and materials. In this event, the Error *A* mean square could be smaller than the Error *B* mean square. Also, if competition is present among the speus within the same whole plot, the Error *B* mean square would contain a component of variance due to competition that would not enter into the Error *A* mean square. This could make Error *B* larger than the Error *A* mean square. Another situation where this could occur is when there is more genetic variation within whole plots than among whole plots. It is also possible owing to lack of symmetry of the distribution of random split plot effects that a transformation of the responses may be required in order to have Error *B* less than Error *A*. Numerical examples can easily be constructed where Error *B* is considerably larger than Error *A*.

1.11. REPEATED MEASURES EXPERIMENTS

Some authors, for example, Kirk (1968, Chapter 8), consider a repeated measures experiment as a split-plot-designed experiment. There are two kinds of repeated measures experiments, viz., the same treatment is repeated *b* times on a single subject or the *b* treatments of factor *B* are applied sequentially to a subject over *b* periods. The latter type of experiment is known as a cross-over-designed experiment. It is this type of repeated measures experiment that has been confused with split-plot-designed experiments. It is inappropriate to consider the cross-over-designed experiment as a split plot experiment. One reason is that there are several kinds of treatment effects in a cross-over experiment, whereas, there is only one kind of treatment effect for split plot experiments. A cross-over experiment may have the direct effect of a treatment in the period in which it was applied, a carryover effect in the periods after it has been applied, a continuing effect, and/or a permanent effect. A split plot experiment has only the direct effect of the treatment. Also the treatment design is different for a cross-over experiment as certain sequences of treatments on a subject are used for a cross-over design, whereas the treatments in a split plot design appear in a random order. The complexity of the nature of treatment effects and the statistical design in a cross-over experiment makes it prudent to consider this class of designs as an entity in itself. Therefore, this type of designed experiment should not be confused with split-plot-designed experiments.

1.12. PRECISION OF CONTRASTS

The average overall precision of the contrasts in a standard split plot design is the same as that for a randomized complete block design of the *ab* treatment combinations. The precision of whole plot treatment contrasts, factor *A*, is usually less than or equal to what it would be for a randomized complete block design. The gain in precision is obtained for the split plot treatments, factor *B*, and for the interaction effects. Thus, if less precision is required for factor *A* treatments and

more for factor B and interaction effects, the split plot design is admirably suited for this situation. Another reason that a split plot design may be selected is that larger experimental units are required for factor A treatments than for factor B treatments. For example, fertilizer and irrigation treatments require larger experimental units than do treatments like varieties, pesticides, etc.

Federer (1955, page 274) presents a measure of the efficiency for split plot treatments (Also, see Kempthorne, 1952, Section 19.4). Using this measure, the precision of the split plot treatments, factor B, and of the $A \times B$ interactions is

$$\frac{(a-1)(\sigma_\varepsilon^2 + b\sigma_\delta^2) + \mathrm{a(b-1)}\sigma_\varepsilon^2}{\mathrm{(ab-1)}\sigma_\varepsilon^2} = 1 + \frac{b(a-1)\sigma_\delta^2}{(ab-1)\sigma_\varepsilon^2}, \tag{1.27}$$

and the precision is estimated by,

$$\frac{(a-1)\mathrm{Error}\,A + a(b-1)\mathrm{Error}\,B}{(ab-1)\mathrm{Error}\,B}. \tag{1.28}$$

The precision of the whole plot treatment effects, factor A, is given by,

$$\frac{(a-1)(\sigma_\varepsilon^2 + b\sigma_\delta^2) + a(b-1)\sigma_\varepsilon^2}{(ab-1)(\sigma_\varepsilon^2 + b\sigma_\delta^2)} = \frac{(a-1)b\sigma_\delta^2 + (ab-1)\sigma_\varepsilon^2}{(ab-1)(\sigma_\varepsilon^2 + b\sigma_\delta^2)}, \tag{1.29}$$

and it is estimated by,

$$\frac{(a-1)\mathrm{Error}\,A + a(b-1)\mathrm{Error}\,B}{(ab-1)\mathrm{Error}\,A}. \tag{1.30}$$

If the variance component σ_δ^2 is equal to zero, the precision is equal to one in both cases.

For the numerical example in Section 1.7, Example 1.2 with $a = 8$ and $b = 4$, the estimated precision for the split plot treatments, seed treatments, and interaction from Equation (1.28) is,

$$\frac{(8-1)(98.38) + 8(4-1)(24.23)}{\{8(4)-1\}(24.23)} = 1.69,$$

that is, a 69% increase over conducting the experiment as a randomized complete block design. The estimated precision for the whole plot treatments, guayule genotypes, from equation (1.30) is,

$$\frac{(8-1)(98.38) + 8(4-1)(24.23)}{\{8(4)-1\}(98.38)} = 0.42,$$

that is, a 58% loss over using a randomized complete block experiment design.

1.13. PROBLEMS

Problem 1.1. For the data of Example 1.1

(i) Analyze the data and use residual diagnostic plots to assess the equal variance and normality assumptions.
(ii) Use a multiple comparison procedure to test significance of pairs of means.
(iii) Redo (i) and (ii) for log transformed data.

Problem 1.2. For the data of Example 1.2

(i) Using the square root, transform the data, and perform analysis.
(ii) Use the arcsine transformation and obtain an analysis of the data.
(iii) Are there any differences in interpretation from these analyses?

Problem 1.3. Mazur (2005) presents several data sets for split-plot-designed experiments. For one of the experiments, four rats, R1, R2, R3, and R4, represented the four blocks or replicates, two whole plot treatments representing two time levels, long (20 s) and short (10 s), and four time intervals between stimuli, B1, B2, B3, and B4, were the split plot treatments. The terminal link entries per hour to the stimuli were:

	R1		R2		R3		R4	
Condition	Long	Short	Long	Short	Long	Short	Long	Short
B1 = 60s	42.8	48.5	51.7	60.0	45.7	49.3	56.8	59.7
B2 = 30s	108.8	53.9	97.9	51.9	86.9	50.8	103.9	56.8
B3 = 15s	100.4	43.7	198.3	54.4	161.4	53.5	211.1	55.7
B4 = 2s	59.8	51.3	899.1	1.9	176.1	51.6	614.9	50.1

(i) Give a linear model for an analysis of these data and state assumptions used.
(ii) Obtain an analysis for these data and interpret the results.
(iii) Use residual diagnostic plots to assess the equal variance and normality assumptions.
(iv) If the assumptions are violated, find a transformation of the data that is suitable to obtain more variance homogeneity.

Problem 1.4. The data for this problem were taken from Mazur (2005). A split plot designed experiment on four pigeons, P1, P2, P3, and P4, as the four blocks was used. Two delay intervals, long (20 s) and short (10 s) represented the whole plot treatments. The eight split plot treatments, B1, B2, B3, B4, B5, B6, B7, and B8, were in a two by four factorial treatment arrangement. The two levels of one factor, say F, were independent, ind, and dependent, dep, and the four levels of the second factor

were variations of presenting the four levels of the second factor, T. The levels of T are those described in Problem 1.3. The terminal entry rates per hour are given in the table below:

	P1		P2		P3		P4	
Condition	Long	Short	Long	Short	Long	Short	Long	Short
B1 = 60sind	31.5	62.3	42.7	52.9	40.4	61.6	5.9	61.8
B2 = 30sind	14.7	67.3	42.1	57.3	19.3	61.2	5.6	57.1
B3 = 15sind	12.2	63.1	42.1	61.2	22.0	56.5	0.8	57.4
B4 = 2sind	8.1	54.8	43.0	60.2	42.0	54.2	8.9	61.1
B5 = 60sdep	38.2	40.0	45.7	46.2	37.6	37.6	25.7	25.3
B6 = 30sdep	62.3	30.4	77.1	39.0	59.1	29.4	31.7	16.4
B7 = 15sdep	104.2	25.2	140.9	34.8	88.6	21.9	28.6	7.5
B8 = 2sdep	124.3	4.1	339.0	11.7	178.8	6.6	69.8	2.4

(i) Obtain an analysis of the data.

(ii) Is variance heterogeneity a problem?

(iii) Obtain an analysis omitting P4 data. Does this change the results?

(iv) Discuss the results of your various analyses and compare the results on rats in the previous problem with those on pigeons.

Problem 1.5. For the data given as Example 2 in Section 2

(i) Write a SAS/PROC GLM code for obtaining an analysis of variance and means.

(ii) Prepare a graph of the corn genotype by district interaction. Is there a significant district by corn genotype interaction?

(iii) Compute the residuals and perform a diagnostic plot to assess the variance homogeneity and normality assumptions.

Problem 1.6. For the data of Example 4 in Section 2

(i) Perform a multiple comparisons procedure using the lsd, the hsd, and the esd methods for the 10 combinations of alfalfa and no alfalfa with the five bromegrass strains. Are there significant differences among the pairs of means?

(ii) Compute Tukey's one degree of freedom for non-additivity. Is there an indication of non-additivity?

1.14. REFERENCES

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APPENDIX 1.1. EXAMPLE 1.1 CODE

The following is the SAS PROC/GLM code for obtaining an analysis of the data for Example 1.1:

```
Data spex1;

input Y R A B; /*Y = yield, R = block, A = planting method,
B = cultivation method*/

datalines;

82.8 1 1 1
46.2 1 1 2
78.6 1 1 3

...

65.6 4 4 3
63.3 4 4 4

;run;

Proc GLM;

Class R A B;
Model Y = R A R*A B A*B;
Lsmeans A B A*B;
Test H = A E = A*R;

Run;
```

The following is an abbreviated form of the output for the above code for Example 1.1:

```
Output
Dependent Variable: Y
```

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	4905.738750	181.694028	10.75	<.0001
Error	36	608.478750	16.902187		
Corrected Total	63	5514.217500			

R-Square	Coeff Var	Root MSE	Y Mean
0.889653	6.215594	4.111227	66.14375

Source	DF	Type I SS	Mean Square	F Value	Pr > F
R	3	223.808750	74.602917	4.41	0.0096
A	3	194.561250	64.853750	3.84	0.0176
R*A	9	158.242500	17.582500	1.04	0.4284
B	3	4107.383750	1369.127917	81.00	<.0001
A*B	9	221.742500	24.638056	1.46	0.2012

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	223.808750	74.602917	4.41	0.0096
A	3	194.561250	64.853750	3.84	0.0176
R*A	9	158.242500	17.582500	1.04	0.4284
B	3	4107.383750	1369.127917	81.00	<.0001
A*B	9	221.742500	24.638056	1.46	0.2012

Least Squares Means

A	Y LSMEAN
1	68.2937500
2	64.0625000
3	64.8437500
4	67.3750000

B	Y LSMEAN
1	71.3875000
2	52.2875000
3	70.7062500
4	70.1937500

A	B	Y LSMEAN
1	1	75.3750000
1	2	52.1000000
1	3	72.2250000
1	4	73.4750000
2	1	72.3000000
2	2	51.3500000
2	3	68.0000000
2	4	64.6000000

```
3    1    67.3250000
3    2    50.4500000
3    3    71.2500000
3    4    70.3500000
4    1    70.5500000
4    2    55.2500000
4    3    71.3500000
4    4    72.3500000
```

```
Dependent Variable: Y

Tests of Hypotheses Using the Type III MS for R*A as an Error Term

Source              DF    Type III SS  Mean Square  F Value    Pr > F
A                    3   194.5612500   64.8537500     3.69   0.0557
```

APPENDIX 1.2. EXAMPLE 1.2 CODE

A SAS PROC/GLM code for obtaining an analysis for the data of Example 1.2 is given below. The code for an analysis for each whole plot data set is obtained by using IF and THEN statements such as "IF A $>$ 1 THEN DELETE; and IF A $<$ 1 THEN DELETE;" to obtain an analysis of the data for genotype 1. An analysis is obtained for the entire data set using the following code:

```
Data spex2;

Input Y R A B; /*Y = count, R = block, A = genotype, B = seed
treatment*/

Datalines;

12  1   0   1
13  1   0   2
66  1   0   0

...

11  3   1   2
15  3   1   3
;

Proc GLM;

Class R A B ;
Model Y = R A R*A B A*B;
Lsmeans A B A*B;
Test H = A E = R*A;

run;
```

An abbreviated form of the output from running the above code is given below:

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	47	35573.40625	756.88098	31.24	<.0001
Error	48	1162.83333	24.22569		
Corrected Total	95	36736.23958			

R-Square	Coeff Var	Root MSE	Y Mean
0.968346	19.45279	4.921960	25.30208

Source	DF	Type I SS	Mean Square	F Value	Pr > F
R	2	38.58333	19.29167	0.80	0.4568
A	7	763.15625	109.02232	4.50	0.0006
R*A	14	1377.25000	98.37500	4.06	0.0001
B	3	30774.28125	10258.09375	423.44	<.0001
A*B	21	2620.13542	124.76835	5.15	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	2	38.58333	19.29167	0.80	0.4568
A	7	763.15625	109.02232	4.50	0.0006
R*A	14	1377.25000	98.37500	4.06	0.0001
B	3	30774.28125	10258.09375	423.44	<.0001
A*B	21	2620.13542	124.76835	5.15	<.0001

Least Squares Means

A	Y LSMEAN
0	24.6666667
1	26.8333333
2	28.8333333
3	21.0000000
4	21.9166667
5	28.1666667
6	23.2500000
7	27.7500000

B	Y LSMEAN
0	55.8333333
1	13.9166667
2	20.0416667
3	11.4166667

A	B	Y LSMEAN
0	0	66.3333333
0	1	11.6666667
0	2	12.3333333
0	3	8.3333333
1	0	63.3333333

1	1	18.3333333
1	2	14.3333333
1	3	11.3333333
2	0	65.0000000
2	1	12.6666667
2	2	26.3333333
2	3	11.3333333
3	0	50.3333333
3	1	10.0000000
3	2	14.0000000
3	3	9.6666667
4	0	49.3333333
4	1	16.3333333
4	2	10.3333333
4	3	11.6666667
5	0	58.0000000
5	1	8.0000000
5	2	29.6666667
5	3	17.0000000
6	0	46.3333333
6	1	14.6666667
6	2	22.0000000
6	3	10.0000000
7	0	48.0000000
7	1	19.6666667
7	2	31.3333333
7	3	12.0000000