Interpreting the analysis of a factorial experiment can be difficult when a significant interaction is present because then the main effects may not be interpreted separately from the interaction. This pamphlet will explore how to interpret main effects in the presence of a significant interaction. We will discuss how to interpret main effects with the help of interaction plots through two separate two-way factorial experiment examples: (i) when a significant interaction is present and one or both main effects are significant; and (ii) when a significant interaction is present and both main effects are not significant.

1. Concepts

Consider a completely randomized two-way factorial experiment to study the effects of FERTILIZER and tree SPECIES on tree height, where both FERTILIZER and SPECIES are fixed factors. The data set is shown in the appendix with two replicates for each combination of FERTILIZER and SPECIES. Table 1 summarizes the data set with average tree heights (cell means). With the help of this table, we will define a few concepts that are important to understand interactions.

Table 1. Data layout showing average tree heights (cell means).

<table>
<thead>
<tr>
<th>FERTILIZER (F)</th>
<th>SPECIES (S)</th>
<th>Mean (Main effect of F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>83.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>60.50</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>41.00</td>
</tr>
<tr>
<td>Mean (Main effect of F)</td>
<td></td>
<td>61.50</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>88.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>42.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>37.50</td>
</tr>
<tr>
<td>Mean (Main effect of S)</td>
<td></td>
<td>55.83</td>
</tr>
<tr>
<td>Mean (Main effect of S)</td>
<td></td>
<td>85.50</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>51.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>39.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>58.67</td>
</tr>
</tbody>
</table>

Main effect: the effect that reflects the general or average effect of a factor. Main effects are revealed by the variation among the row or column means, also known as marginal means (since the means are in the margins of the table). For example, the main effects of SPECIES are calculated as 85.50, 51.25, and 39.25. If these three numbers are shown to be significantly different from one another using the F-test in ANOVA, we would conclude that the SPECIES effect was statistically significant.

1 It is not meaningful to interpret an interaction that includes a random factor since it would be the variance that is of interest rather than the means. For a discussion about interactions which include a random factor, see Biom. Info. Pamph. #48.
2 Technically speaking, most statistical textbooks define main effects as the difference between the marginal means and the grand mean. For example, the main effects of SPECIES would be calculated as 85.50 – 58.67 = 26.83, 51.25 – 58.67 = 7.42, and 39.25 – 58.67 = 19.42. This discrepancy in definition does not affect the following discussion.
**Simple effect:** the effect that a factor has at a given level of another variable. Simple effects are revealed by examining the cell means within the body of the table either within a row or within a column. For example, the simple effects of FERTILIZER at level 2 of SPECIES are 60.50 and 42.00. We would conclude that FERTILIZER has significant simple effects at SPECIES = 2 if these two numbers are statistically different. (See Figure 2 and its interpretation for how to test if the two simple effects are significantly different.)

**Interaction:** Two variables interact when the simple effects of one variable depend on the levels of the second variable (Keppel, 1982). Interactions can be revealed by interaction plots.

**Interaction Plot:** This is a plot of cell means of the response variable (e.g., tree height) versus levels of one variable (e.g., FERTILIZER) for each level of another variable (e.g., SPECIES).

2. Example where interaction is present and one or both main effects are significant

The ANOVA table (Table 2) indicates that the 2-way interaction, S*F, is significant. Therefore the main effects may **not** be interpreted separately from the interaction even though the p-value for SPECIES is very small. To find out whether the main effects have a simple interpretation, let’s examine the interaction plots shown in Figures 1 and 2.

**Table 2.** ANOVA Table for the example.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECIES, S</td>
<td>2</td>
<td>4608.17</td>
<td>2304.08</td>
<td>99.46</td>
<td>0.0001</td>
</tr>
<tr>
<td>FERTILIZER, F</td>
<td>1</td>
<td>96.33</td>
<td>96.33</td>
<td>4.16</td>
<td>0.088</td>
</tr>
<tr>
<td>S*F</td>
<td>2</td>
<td>283.17</td>
<td>141.58</td>
<td>6.11</td>
<td>0.036</td>
</tr>
<tr>
<td>ERROR</td>
<td>6</td>
<td>139.00</td>
<td>23.17</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Figure 1 illustrates the interaction between SPECIES and FERTILIZER level, while the three lines are not parallel, they do not cross one another. This is called an **ordinal interaction** from the perspective of the SPECIES factor (Keppel, 1973). Although the difference between any two SPECIES’ means changes with the fertilizer level, the ranking of the SPECIES does not change. Notice that the line representing SPECIES 1 is above the other two lines. This suggests that the height of SPECIES 1 was consistently largest for both levels of FERTILIZER. Also, the response is always lowest for SPECIES 3 with SPECIES 2 being intermediate for both levels of FERTILIZER. The main effect of SPECIES can be interpreted separately from FERTILIZER as just described. In other words, the p-value of 0.0001 in Table 2 can be interpreted as a highly significant difference among SPECIES.

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3 To be exact, simple effects are usually calculated as the differences between the cell means and the marginal mean for a particular level. For example, the simple effect of FERTILIZER at level 2 of SPECIES would be computed as 60.50 - 51.25 = 9.25 and 42.00 - 51.25 = -9.25. Again, this difference in definition is merely a technical point.
Figure 1. Interaction plot for the example — to display the effects of SPECIES.

Figure 2. Interaction plot for the example — to display the effects of FERTILIZER.
Figure 2 is an alternative interaction plot. In Figure 2, not only are the two “curves” not parallel, but they also cross over one another. The “curve” for F=1 is not always above or below that of F=2. This is called a disordinal interaction from the perspective of the factor FERTILIZER (Keppel, 1973). In this case, effects of FERTILIZER cannot be interpreted separately from the interaction, but can only be interpreted for each particular level of SPECIES.

To interpret the effects of FERTILIZER at each individual level of SPECIES, we can compare the simple effects of FERTILIZER for each level of SPECIES. The Least Significant Difference (LSD) with a Bonferroni correction can be used to identify whether two simple effects are significantly different. For the ANOVA in Table 2, the LSD for comparing 3 pairs of simple effects at an overall significance level of $\alpha = 0.05$, is computed by:

$$LSD = t_{\alpha/2, df} \sqrt{2MSE} \left/ \sqrt{r} \right. = t_{0.05/6, 6} \sqrt{2 \times 23.1667} \left/ \sqrt{2} \right. = 3.29 \times 4.813 = 15.8,$$

where $k$ is the number of comparisons to be made, $r$ is the number of observations used to calculate each simple effect, $\alpha$ is the significance level, $MSE$ is the mean square error shown in the ANOVA table, $df$ is the degrees of freedom associated with the $MSE$, and $t_{\alpha/6, df}$ is the $(1 - \frac{\alpha}{6}) \times 100th$ percentile of the student t-distribution$^4$ with degrees of freedom of $df$.

Any two means (simple effects in this case) whose difference exceeds $LSD$ are declared significantly different (Cochran and Cox, 1957). In Figure 2, with $LSD = 15.8$, for $SPECIES = 1$, FERTILIZER = 1 is not significantly different from FERTILIZER = 2. For $SPECIES = 2$, FERTILIZER = 1 is significantly more effective than FERTILIZER = 2. For $SPECIES = 3$, FERTILIZER = 1 is not significantly different from FERTILIZER = 2. The average FERTILIZER effect over all levels of SPECIES are not significant as the p-value = 0.0875 (from Table 2) indicates.

When an ordinal interaction is present as illustrated in Figure 1, then the main effects may still be interpreted separately. The ordinality of an interaction depends on how the results are plotted (Figure 1 is ordinal but Figure 2 is disordinal). For this reason, if there is an interaction present, it is wise to plot the data both ways before the significant main effects are interpreted. If ordinality exists, the main effect may be interpreted as a main effect. If it does not, the main effect cannot be interpreted separately from the interaction but can only be interpreted individually for each particular level of the other factor (Keppel, 1973).

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$^4$ We use $t_{\alpha/6, df}$ instead of $t_{\alpha, df}$ to compute the LSD. This is because the Bonferroni correction is applied to adjust the significance level so that the overall significance level is $\alpha$. Generally, when $k$ comparisons are to be made, we set each individual comparison at level $\alpha / 2k$, so that the overall significance level will be no more than $\alpha$ (Milliken and Johnson, 1992).
### 3. Example where interaction is significant but main effects are not

Consider the same two-way factorial design in the example, but with modified data (four replicates for each combination of FERTILIZER and SPECIES instead of two).

#### Table 3. ANOVA Table for the modified example data.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECIES, S</td>
<td>2</td>
<td>112.00</td>
<td>56.00</td>
<td>3.05</td>
<td>0.072</td>
</tr>
<tr>
<td>FERTILIZER, F</td>
<td>1</td>
<td>24.00</td>
<td>24.00</td>
<td>1.31</td>
<td>0.27</td>
</tr>
<tr>
<td>S*F</td>
<td>2</td>
<td>144.00</td>
<td>72.00</td>
<td>3.93</td>
<td>0.038</td>
</tr>
<tr>
<td>ERROR</td>
<td>18</td>
<td>330.00</td>
<td>18.33</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 3 shows that the S*F interaction is significant, but FERTILIZER and SPECIES are not (at \( \alpha = 0.05 \)). What does this mean? The significant interaction S*F suggests that the simple effects of SPECIES (or FERTILIZER) are different at different levels of FERTILIZER (or SPECIES). The significant interaction and nonsignificant tests of main effects suggests that, in combination, the factors SPECIES and FERTILIZER do have a significant effect on tree height, while the main effects of SPECIES and FERTILIZER require further examination.

To see how the combinations of SPECIES and FERTILIZER affect tree height, we examine the interaction plots shown in Figures 3 and 4. In Figure 3, there are 3 pairs of means to be compared, so we have

\[
LSD = t_{0.05/6,18} \frac{\sqrt{2 \times 18.3}}{\sqrt{4}} = 2.64 \times \frac{\sqrt{18.3}}{\sqrt{2}} = 7.9.
\]

With \( LSD = 7.9 \), the plot in Figure 3 shows that for SPECIES = 1, the response is significantly higher for FERTILIZER = 2; for SPECIES = 2 and 3, FERTILIZER cell means are not significantly different. We cannot conclude that one level of FERTILIZER is more effective than the other over all levels of SPECIES.

Figure 4 is an alternative plot of the interaction plot in Figure 3. In Figure 4, there are 6 pairs of simple effects to be compared. For FERTILIZER = 1, with

\[
LSD = t_{0.05/12,18} \frac{\sqrt{2 \times 18.3}}{\sqrt{4}} = 2.96 \times \frac{\sqrt{18.3}}{\sqrt{2}} = 8.96,
\]

SPECIES = 1 has a significantly lower response than SPECIES = 3, and a marginally lower response than SPECIES = 2, while SPECIES = 2 is not significantly different from SPECIES = 3. At FERTILIZER = 2, all SPECIES levels are not significantly different. Again, no conclusion can be made as to which level of SPECIES is more effective over both levels of FERTILIZER. The three species appear to have a more variable response to FERTILIZER 1 than to FERTILIZER 2, suggesting that more research is required to elucidate the effect of FERTILIZER 1 on tree growth.
**Figure 3.** Interaction plot for the modified example — to display the effects of FERTILIZER.

**Figure 4.** Interaction plot for the modified example — to display the effects of SPECIES.
In summary, when an interaction is significant, although the main effects are not, the factors do affect the results, but only in combination with the other factor. In other words main effects cannot be interpreted separately from the interaction. However, you can interpret treatment effects separately for each level of the other factor.

Prepared by:

References:

Susan replaced Vera Sit while she was on maternity leave.
Appendix:

SAS Program to produce one of the ANOVA tables and two interaction plots.

```
TITLE "ANOVA TABLE AND INTERACTION PLOTS";
DATA RAW; /** INPUT DATA SET RAW **/
   INPUT S F Y;
   CARDS;
   1 1 78
   1 1 88
   1 2 92
   1 2 84
   2 1 58
   2 1 63
   2 2 44
   2 2 40
   3 1 37
   3 1 45
   3 2 39
   3 2 36
;
PROC GLM DATA=RAW;
   CLASS S F;
   MODEL Y=S|F; /* TO FIT ANOVA MODEL Y = S F S*F. */
   MEANS S/SNK; /* TO LIST THE MEAN VALUES OF Y FOR EACH LEVEL OF FACTOR S */
   /* AND TO TEST IF THE MEAN VALUES ARE THE SAME FOR ALL THE */
   /* THREE LEVELS OF SPECIES WITH STUDENT-NEWMAN-KEULS RANGE */
   /* TEST. THE "MEANS" STATEMENT IS ONLY GOOD FOR BALANCED */
   /* DESIGNS. */
   MEANS F S*F/SNK;
   LSMEANS S*F/OUT=CELLMEAN; /* TO LIST MEAN VALUES OF Y FOR EACH LEVEL OF */
   /* S*F AND TO OUTPUT THE CELLMEANS INTO FILE */
   /* ‘CELLMEAN’. IT IS PARTICULARLY USEFUL FOR */
   /* EXAMINING INTERACTIONS AND WORKS FOR */
   /* BOTH UNBALANCED AND BALANCED DESIGNS. */
RUN;
PROC PLOT DATA=CELLMEAN; /* TO DESIGNATE DATA SET "CELLMEAN" TO BE USED */
   /* TO PRODUCE THE FOLLOWING INTERACTION PLOTS. */
   PLOT LSMEAN*S=F; /* TO PRODUCE AN INTERACTION PLOT TO DISPLAY */
   /* THE F EFFECTS. */
   PLOT LSMEAN*F=S;
RUN;
QUIT;
```