Two-Factor Analysis of Variance (continued)

A plant pathologist was studying the effect of soybean cyst nematode infection on the activity level of a soybean gene suspected to play a role in plant defense against pathogen attacks. A total of 24 soybean plants were used in the experiment. Half of the plants were randomly selected to be inoculated with soybean cyst nematodes. The other half were mock inoculated with a control substance. The activity level of the gene was measured immediately after inoculation for 4 plants in each group. The activity level of the gene was measured 12 hours after inoculation for another 4 plants in each group. The activity level of the gene was measured 24 hours after inoculation for the 4 remaining plants in each group. The treatments are summarized below.

Treatment	Inoculation	Time Point	Number of Plants	Mean Activity Level
1	control	0	4	9.50
2	control	12	4	10.00
3	control	24	4	10.75
4	nematodes	0	4	9.75
5	nematodes	12	4	13.00
6	nematodes	24	4	17.25

1. This is an example of a two-factor experiment. Name the factors and the levels of each factor.

2. For two-factor experiments with two levels for each factor, we learned how to test for interaction between factors by testing whether a linear combination of treatment means was significantly different from zero. When one or more factors has more than two levels, we will test for interaction using the F-test provided in the *Type III Sum of Squares* portion of the SAS output. If there are a levels of one factor and b levels of the other factor, the F-test for interaction has numerator degrees of freedom $(a-1)(b-1)$ and denominator degrees of freedom $(n-ab)=(n-I)$ where n is the total sample size and I is the number of treatments. Note that the denominator degrees of freedom $(n-I)$ matches the degrees of freedom for error because the denominator of the F-statistic is MSE. Give the numerator and denominator degrees of freedom for the F-test for interaction in this example.

3. For two-factor experiments with two levels for each factor, we learned how to test for differences between the levels of one factor while averaging over the levels of the other factor by testing whether a linear combination of treatment means was significantly different from zero. This same strategy will work for any factor with only two levels, regardless of how many levels the other factor has. Test for a difference between control and treatment with nematodes by averaging over the levels of time in this example. To conduct the test, you need to know that $MSE=2.736$.

4. When a factor has more than two levels, it is not possible to test for differences among levels of the factor by testing the significance of only one linear combination of means. Thus when a factor has more than two levels, we will test for the presence of any differences among the levels of the factor (averaging over the levels of the other factor) by using the F-test provided in the *Type III Sum of Squares* portion of the SAS output. If there are a levels of factor A and b levels of the other factor B, the F-test for differences among the levels of factor A has numerator degrees of freedom $(a-1)$, and the F-test for differences among the levels of factor B has numerator degrees of freedom $(b-1)$. Both tests have denominator degrees of freedom $(n-ab)=(n-I)$ because the denominator of the F-statistic is MSE. Compute treatment averages for each level of the time factor in this example, and give the numerator and denominator degrees of freedom for testing whether there are any significant differences among these averages.

5. Examine the SAS code and output below. Is there evidence that this gene plays a role in defense against pathogen attack? Explain.

```
proc glm;
  class inoc time;
  model y=inoc time inoc*time;
  lsmeans inoc time;
  lsmeans inoc*time / slice=time;
  lsmeans inoc*time / slice=inoc;
  estimate 'nematode - control at time 0' inoc -1 1 inoc*time -1 0 0 1 0 0;
  estimate 'nematode - control at time 12' inoc -1 1 inoc*time 0 -1 0 0 1 0;
  estimate 'nematode - control at time 24' inoc -1 1 inoc*time 0 0 -1 0 0 1;
run;
```

```
The GLM Procedure

         Class Level Information

Class         Levels    Values
inoc               2    control nematode
time               3    0 12 24

Number of observations    24

Dependent Variable: y (activity level)

                                        Sum of
Source                      DF         Squares     Mean Square    F Value    Pr > F
Model                        5     179.7083333      35.9416667      13.14    <.0001
Error                       18      49.2500000       2.7361111
Corrected Total             23     228.9583333

R-Square     Coeff Var      Root MSE        y Mean
0.784895      14.12771      1.654119      11.70833

Source                      DF       Type I SS     Mean Square    F Value    Pr > F
inoc                         1     63.37500000     63.37500000      23.16    0.0001
time                         2     77.08333333     38.54166667      14.09    0.0002
inoc*time                    2     39.25000000     19.62500000       7.17    0.0051
```

Source	DF	Type III SS	Mean Square	F Value	Pr > F
inoc	1	63.37500000	63.37500000	23.16	0.0001
time	2	77.08333333	38.54166667	14.09	0.0002
inoc*time	2	39.25000000	19.62500000	7.17	0.0051

Least Squares Means

inoc	y LSMEAN
control	10.0833333
nematode	13.3333333

time	y LSMEAN
0	9.6250000
12	11.5000000
24	14.0000000

inoc	time	y LSMEAN
control	0	9.5000000
control	12	10.0000000
control	24	10.7500000
nematode	0	9.7500000
nematode	12	13.0000000
nematode	24	17.2500000

inoc*time Effect Sliced by time for y

time	DF	Sum of Squares	Mean Square	F Value	Pr > F
0	1	0.125000	0.125000	0.05	0.8332
12	1	18.000000	18.000000	6.58	0.0195
24	1	84.500000	84.500000	30.88	<.0001

inoc*time Effect Sliced by inoc for y

inoc	DF	Sum of Squares	Mean Square	F Value	Pr > F
control	2	3.166667	1.583333	0.58	0.5707
nematode	2	113.166667	56.583333	20.68	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
nematode - control at time 0	0.25000000	1.16963907	0.21	0.8332
nematode - control at time 12	3.00000000	1.16963907	2.56	0.0195
nematode - control at time 24	6.50000000	1.16963907	5.56	<.0001