Solution to Exercise 9.8 (Version 1, 1/8/15)

from Statistical Methods in Biology: Design & Analysis of Experiments and Regression (2014) S.J. Welham, S.A. Gezan, S.J. Clark & A. Mead. Chapman & Hall/CRC Press, Boca Raton, Florida. ISBN: 978-1-4398-0878-8

Exercise 9.8 (Data: courtesy S. Foster, Rothamsted Research)

An experiment assessed the response of two aphid clones to a foliar insecticide applied to cabbage plants. The experiment used two simulators, each containing six plants in individual pots. All plants in one simulator were sprayed with the insecticide and all plants in the other were sprayed with water only (control). Two weeks after spraying adult aphids were placed onto the plants using clip cages. Two clip cages were attached to each plant, one containing three aphids of a clone susceptible to the insecticide, the other containing three aphids of a moderately resistant clone. The number of nymphs produced by the adults in each clip cage was recorded after two days. The experiment was then repeated using two new simulators. File SIMULATOR.DAT contains the unit numbers (*ID*), structural factors (Expt, DSimulator, Plant, DCage), explanatory factors (Treatment, Clone) and the nymph counts (variate *Nymphs*). Determine the structural and explanatory components for this experiment, write down the full model in symbolic form and state the experimental units for the insecticide and clone treatments. Analyse the data and verify that the explanatory terms are tested in the correct strata. Form and interpret the predictive model. (We re-visit these data in Exercise 16.1.)

Data 9.8 (SIMULATOR.DAT)

Number of nymphs produced in two days by two aphid clones (S = susceptible, R = resistant) where plants had been treated two weeks earlier with insecticide (I) or water (control, C) (Expt = Experiment, Sim = Simulator, Plt = Plant, Cage, Trt = Treatment, Cln = Clone, Nym = Number of nymphs):

ID	Expt	Sim	Plt	Cage	Trt	Cln	Nym	ID	Expt	Sim	Plt	Cage	Trt	Cln	Nym
1	1	1	1	1	C	S	21	25	2	1	1	1	C	S	18
2	1	1	1	2	C	R	19	26	2	1	1	2	C	R	21
3	1	1	2	1	C	S	20	27	2	1	2	1	C	S	25
4	1	1	2	2	C	R	16	28	2	1	2	2	C	R	29
5	1	1	3	1	C	S	24	29	2	1	3	1	C	S	26
6	1	1	3	2	C	R	14	30	2	1	3	2	C	R	15
7	1	1	4	1	C	S	25	31	2	1	4	1	C	S	28
8	1	1	4	2	C	R	23	32	2	1	4	2	C	R	21
9	1	1	5	1	C	S	26	33	2	1	5	1	C	S	38
10	1	1	5	2	C	R	27	34	2	1	5	2	C	R	31
11	1	1	6	1	C	S	28	35	2	1	6	1	C	S	30
12	1	1	6	2	C	R	24	36	2	1	6	2	C	R	16
13	1	2	1	1	I	S	23	37	2	2	1	1	I	S	26
14	1	2	1	2	I	R	23	38	2	2	1	2	I	R	31
15	1	2	2	1	I	S	21	39	2	2	2	1	I	S	25
16	1	2	2	2	I	R	21	40	2	2	2	2	I	R	34
17	1	2	3	1	I	S	17	41	2	2	3	1	I	S	11
18	1	2	3	2	I	R	15	42	2	2	3	2	I	R	16
19	1	2	4	1	I	S	17	43	2	2	4	1	I	S	13

20	1	2	4	2	I	R	19	44	2	2	4	2	I	R	27
21	1	2	5	1	I	S	14	45	2	2	5	1	I	S	25
22	1	2	5	2	I	R	23	46	2	2	5	2	I	R	30
23	1	2	6	1	I	S	19	47	2	2	6	1	I	S	22
24	1	2	6	2	I	R	18	48	2	2	6	2	I	R	22

Solution 9.8

First, we consider the structural component. In this experiment the measurement units are clip cages (DCage) and these cages are nested within plants, the aphids in each pair of cages sharing a common host plant (Plant) and with no association between cages on different plants. Plants are nested within simulators (DSimulator), as there is no association between plants in different simulators. Finally, as different simulators are used in each repeat of the experiment, the simulators can be considered as nested within runs (labelled Expt). The overall structural component is therefore a four-level nested structure, written as

Structural component: Expt / DSimulator / Plant / DCage

Next we consider the explanatory component. There are two treatment factors: clone type and insecticide treatment. The two clone types are allocated at random to the two clip cages within each plant, and so the experimental units for this treatment are the Expt.DSimulator.Plant.DCage combinations. All of the plants in each simulator had the same insecticide treatment, so we consider simulators within runs (Expt.DSimulator combinations) as the experimental unit for the insecticide treatment. All four combinations of the two insecticide treatments and two clones are tested in the experiment, with 12 replicates of each combination overall. The explanatory component is crossed and the full set of treatments forms a 2 × 2 factorial set with equal replication. The explanatory component can therefore be written as

Explanatory component: [1] + Treatment*Clone

With the number of nymphs after two days (variate *Nymphs*) as the response, the full model can be written in symbolic notation as

Response variable: *Nymphs*
Explanatory component: [1] + Treatment*Clone
Structural component: Expt / DSimulator / Plant / DCage

The residual plots from this model are shown in Figure S9.8.1. Although these are count data, there is no indication of any variance heterogeneity or skewness and we therefore continue to interpret the analysis on the original count scale.

The multi-stratum ANOVA table from this model is Table S9.8.1. As required, the insecticide treatment effects (term Treatment) are tested against background variability in the Expt.DSimulator stratum, and the clone effects (term Clone) and the interaction (term Treatment.Clone) are tested against the cage-to-cage background variability in the Expt.DSimulator.Plant.DCage stratum. Note that as there are only two simulators used in each run of the experiment, the residual df in the Expt.DSimulator stratum are very small (residual df = 1). There is therefore little power to detect differences between the insecticide treatments even if these are present (see Chapter 10). There is strong evidence of an interaction between the insecticide treatment and clone types ($F_{1,22} = 14.67$), but no evidence of any differences in the main effects ($P > 0.05$).

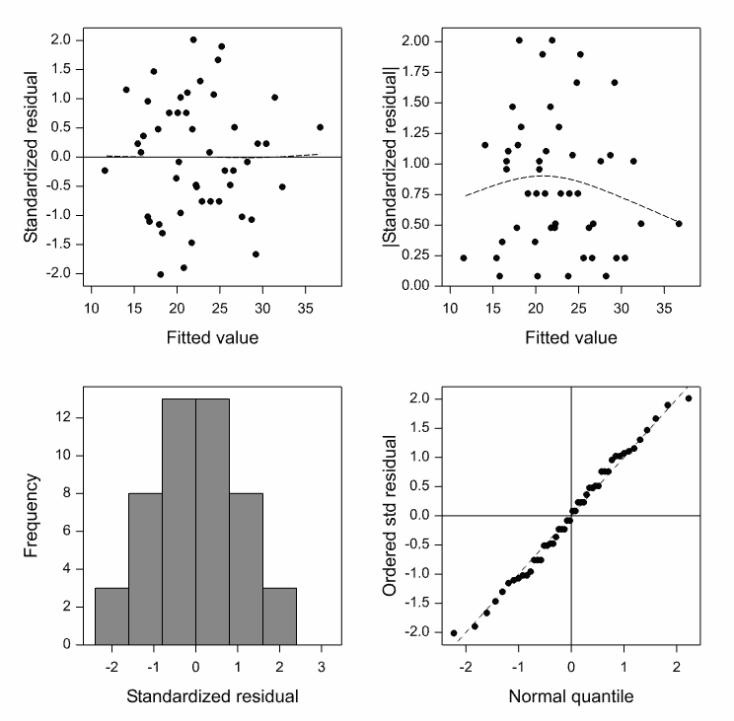

Figure S9.8.1. Composite set of residual plots based on standardized (std) residuals after analysis of aphid nymph counts.

Table S9.8.1 Multi-stratum ANOVA table for nymph counts from a 2 × 2 factorial experiment with four nested structural levels, with clone types allocated to clip cages and insecticide treatments to incubators.

Source of variation	df	Sum of squares	Mean square	Variance ratio	*P*
Expt stratum	1	143.52	143.52	15.621	
Expt.DSimulator stratum					
Treatment	1	58.52	58.52	6.370	0.240
Residual	1	9.19	9.19	0.209	
Expt.DSimulator.Plant stratum	2	879.08	43.95	3.157	
Expt.DSimulator.Plant.DCage stratum					
Clone	1	1.02	1.02	0.073	0.789
Treatment.Clone	1	204.19	204.19	14.666	< 0.001
Residual	22	306.29	13.92		
Total	47	1601.81			

Respecting the principle of marginality, the predictive model must therefore include both main effects as well as the interaction, and so is the same as the explanatory component:

[1] + Treatment*Clone

We therefore produce tables of predicted means classified by both explanatory factors. These predictions are in Table S9.8.2 and Figure S9.8.2. We conclude that there is no evidence of any difference in population mean number of nymphs produced by resistant clones in the absence/presence of insecticide, but strong evidence that the population mean number of nymphs produced by susceptible clones is reduced when insecticide is used.

Table S9.8.2 Predicted means for all combinations of treatment (control or insecticide) and clone type (resistant or susceptible). SED = 1.523 (same treatment with 22 df), 1.388 (different treatments with 5.73 df). LSD = 3.159 (same treatment, 22 df), 3.435 (different treatments, 5.73 df) with $\alpha_s = 0.05$.

	Clone type	
Treatment	Resistant	Susceptible
Control	21.33	25.75
Insecticide	23.25	19.42

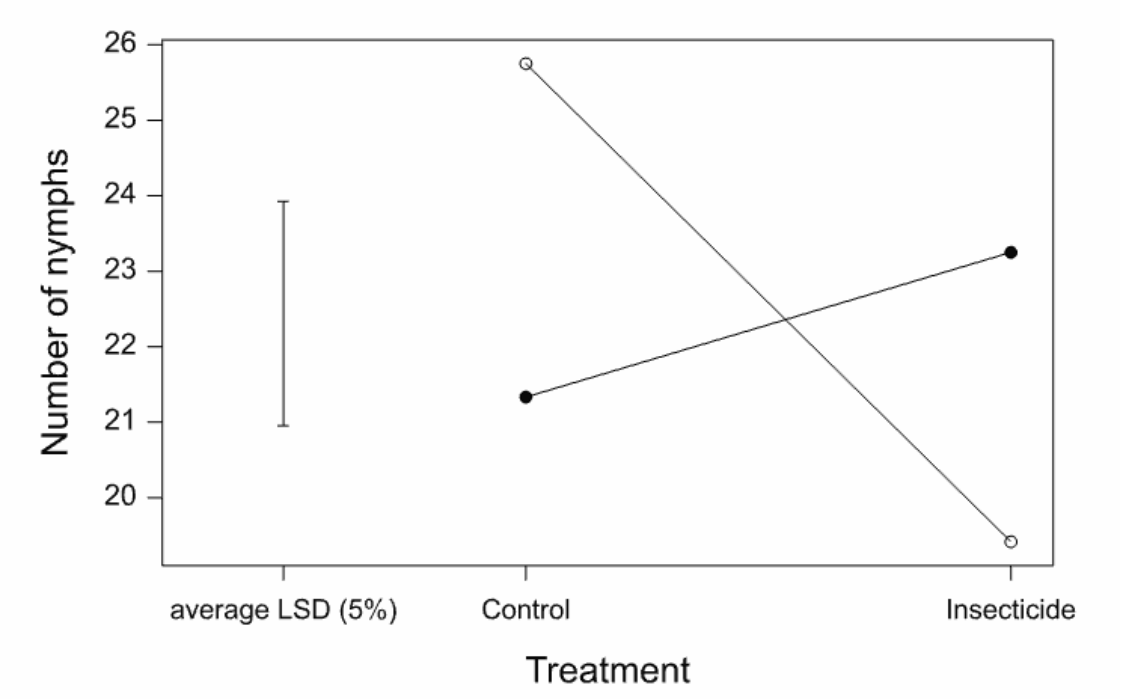

Figure S9.8.2. Predicted population mean numbers of nymphs for resistant (●) and susceptible (○) clones in the absence (Control) or presence of insecticide, with average LSD shown (with $\alpha_s = 0.05$).