**Solution to Exercise 8.2** (Version 1, 26/6/15)

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

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## Exercise 8.2 (Data: courtesy M. Wilkinson, Rothamsted Research)

An experiment designed as a RCBD with three blocks and a  $2 \times 2 \times 2$  factorial structure investigated the effect of three factors and their interactions on the rate of callus growth on wheat seeds. Wheat seeds were placed in separate isolation containers with sets of eight containers, one for each of the eight treatments, kept together in holding trays (factor Tray). The treatment factors were age of the seed ('old' or 'young', factor Age), concentration of growth media (2.5 or 5 mg, factor Conc) and type of growth promoter (Cutlass or Rapier, factor Type). Seeds were weighed (variate *Weight*) after they had been in the media for 15 days. Analyse the seed weights; the data set is in file CALLUS.DAT. Remember to check the model assumptions. What conclusions can you draw from this experiment?

## Data 8.2 (CALLUS.DAT)

Seed weights from experiment using containers (Cont) within trays to investigate the effects of age, type and concentration (Conc) of growth promoter on rate of callus growth.

Tray	Cont	Age	Conc	Type	Weight	Tray	Cont	Age	Conc	Type	Weight
1	1	Old	2.5	Cutlass	0.078	2	5	Young	2.5	Cutlass	0.459
1	2	Old	2.5	Rapier	0.088	2	6	Young	2.5	Rapier	0.203
1	3	Old	5	Cutlass	0.329	2	7	Young	5	Cutlass	0.648
1	4	Old	5	Rapier	0.129	2	8	Young	5	Rapier	0.379
1	5	Young	2.5	Cutlass	0.312	3	1	Old	2.5	Cutlass	0.227
1	6	Young	2.5	Rapier	0.258	3	2	Old	2.5	Rapier	0.129
1	7	Young	5	Cutlass	0.429	3	3	Old	5	Cutlass	0.204
1	8	Young	5	Rapier	0.330	3	4	Old	5	Rapier	0.260
2	1	Old	2.5	Cutlass	0.197	3	5	Young	2.5	Cutlass	0.423
2	2	Old	2.5	Rapier	0.151	3	6	Young	2.5	Rapier	0.222
2	3	Old	5	Cutlass	0.281	3	7	Young	5	Cutlass	0.352
2	4	Old	5	Rapier	0.229	3	8	Young	5	Rapier	0.384

## **Solution 8.2**

The structure of the experiment is a RCBD with trays as blocks, and containers nested within trays are the individual experimental units. The structural component of the model can therefore be written as

Structural component: Tray / Container

The treatments are a  $2 \times 2 \times 2$  factorial set of 8 treatment. The treatments are crossed, and so we have a 3-way crossed structure, which we can write as

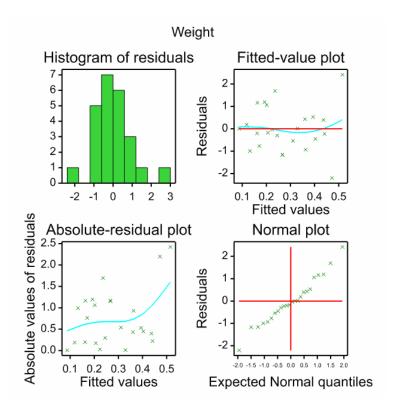
Explanatory component: [1] + Age\*Conc\*Type

With response variate *Weight*, the model can then be written in full in symbolic form as

Response variate: Weight

Structural component: Tray / Container
Explanatory component: [1] + Age\*Conc\*Type

The standardized residuals obtained from this model are plotted in Figure S8.2.1. There are two large residuals corresponding to the largest fitted values (fitted value ~0.5), but there does not appear to be any relationship of variance with fitted value when considered across the full range. The histogram of residuals is approximately bell-shaped and symmetric, and the Normal plot shows a reasonable straight line. We accept these residuals as being consistent with the assumptions underlying the ANOVA, and move on to interpret the ANOVA table.



**Figure S8.2.1**. Residual plots for standardized residuals of seed weights from 3-way crossed model.

The multi-stratum ANOVA table corresponding to the 3-way crossed model is shown in Table S8.2.1 below. The variance ratios give no evidence (P > 0.05) that the three-way interaction, or any of the two-way interactions are present. We conclude that the effect of any one factor is unmodified by the choice made for the other two factors. The variance ratios for the three main effects (Age, Conc and Type) give strong evidence (all with P < 0.01) of differences in seed weights between the two levels of each factor. So there is evidence that all three factors affect seed weight, but they are acting independently. We can therefore summarize the results by looking at the response to each factor separately, as shown in Table S8.2.2.

**Table S8.2.1**. Multi-stratum ANOVA table for 3-way crossed model for seed weights.

Source of variation	df	Sum of squares	Mean square	Variance ratio	P
Tray stratum					
Residual	2	0.02225	0.01113	2.170	
Tray.Container stratum					
Age	1	0.18323	0.18323	35.731	< 0.001
Conc	1	0.06070	0.06070	11.837	0.004
Туре	1	0.05772	0.05772	11.256	0.005
Age.Conc	1	0.00029	0.00029	0.056	0.816
Age.Type	1	0.01114	0.01114	2.172	0.163
Conc.Type	1	0.00053	0.00053	0.104	0.752
Age.Conc.Type	1	0.00234	0.00234	0.456	0.510
Residual	14	0.07179	0.00513		
Total	23	0.40999			

**Table S.8.2.2** Predicted means of seed weight (averaged over other factors) for each age, concentration and type. SED between means within each factor = 0.02923, with 14 df.

Age	Predicted	Conc	Predicted	Type	Predicted
	mean		mean		mean
Old	0.1918	2.5	0.2289	Cutlass	0.3282
Young	0.3666	5.0	0.3295	Rapier	0.2302

From the ANOVA table and the predicted means in Table S8.2.2, we can conclude that the age of the seed has the biggest influence on callus growth, with old seeds weighing 0.175 units less that young seeds at the end of the experiment (SED = 0.0292, df = 14). (We would of course have verified that the old and young seeds had similar weights at the start of the experiment!) The magnitude of the effects of the growth media and promoter are similar, with the higher concentration of growth media giving a 0.101 unit increase in seed weight than the lower concentration (SED = 0.0292, df = 14), and the Cutlass promoter giving 0.098 unit increase in seed weight over Rapier (SED = 0.0292, df = 14).