Solution to Exercise 8.4 (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

Exercise 8.4 (Data: courtesy P. Lutman, Rothamsted Research)

A field experiment to investigate the effect of weed competitors on yield of winter wheat was set up as a RCBD with three blocks of 18 plots. Three weed species were used: chickweed (CW), black-grass (BG) and cleavers (CL). Target weed densities were 0, 40, 80, 160, 320 and 640 plants per m^2 for CW and BG, and 0, 3, 6, 12, 24 and 48 plants per m^2 for CL. However, the weed densities achieved were lower and differed among species. The unit numbers (*ID*), structural factors (Block, Plot), species sown (factor Weed), density achieved (variate *Density*) and the final yields at harvest (variate *Yield*, tonnes/hectare at 85% dry matter) are given in file WEEDCOMPETITION.dat. Consider whether it is appropriate to consider density as crossed with or nested within weed species, and construct a suitable factor for the density treatment. Analyse the data and interpret the tests generated from your ANOVA table. What conclusions can you draw from this trial?

Data 8.4 (WEEDCOMPETITION.DAT)

Plot yields for each combination of block, weed type and average achieved density for each weed type:

Block	Plot	Weed	Density	Yield	Block	Plot	Weed	Density	Yield
1	1	CL	3.8	8.98	2	10	CW	0.0	8.23
1	2	CW	90.3	7.34	2	11	CL	24.9	7.61
1	3	BG	183.0	6.25	2	12	BG	92.8	6.62
1	4	CL	4.1	7.77	2	13	CW	90.3	8.29
1	5	CW	0.0	7.70	2	14	CL	2.1	7.89
1	6	BG	19.9	8.03	2	15	CW	10.9	8.72
1	7	CW	130.0	6.99	2	16	CL	4.1	7.58
1	8	CW	10.9	8.78	2	17	BG	183.0	7.75
1	9	BG	57.7	8.52	2	18	BG	0.0	8.26
1	10	BG	0.0	8.30	3	1	CW	10.9	7.76
1	11	CL	2.1	7.88	3	2	CL	2.1	7.86
1	12	CL	9.6	7.56	3	3	BG	92.8	7.34
1	13	CW	29.8	7.64	3	4	BG	0.0	7.86
1	14	BG	92.8	6.40	3	5	CW	29.8	8.47
1	15	CL	0.0	8.49	3	6	CL	24.9	7.62
1	16	BG	28.8	7.57	3	7	BG	183.0	7.68
1	17	CL	24.9	6.90	3	8	CL	0.0	8.38
1	18	CW	3.8	8.12	3	9	CW	90.3	6.11
2	1	CW	3.8	8.70	3	10	CL	4.1	8.06
2	2	BG	28.8	8.50	3	11	BG	28.8	7.42
2	3	BG	57.7	7.07	3	12	CW	3.8	8.48
2	4	CL	0.0	7.64	3	13	CL	9.6	7.67
2	5	CW	130.0	5.97	3	14	BG	57.7	7.52
2	6	CL	9.6	7.39	3	15	BG	19.9	8.22

Block	Plot	Weed	Density	Yield	Block	Plot	Weed	Density	Yield
2	7	BG	19.9	7.76	3	16	CL	3.8	7.94
2	8	CL	3.8	8.26	3	17	CW	130.0	7.72
2	9	CW	29.8	8.06	3	18	CW	0.0	7.97

Solution 8.4

In the original plan for the experiment, the target plant density for each weed species differed. Unless these densities were chosen to give equivalent yield losses, there is then no correspondence between densities across weed species, and so we would usually consider them as nested within the weed species. In fact, the target plant densities were not achieved and any equivalence would therefore be lost, so there are very good grounds to consider the densities as nested within species. The raw data are shown in Figure S8.4.1.

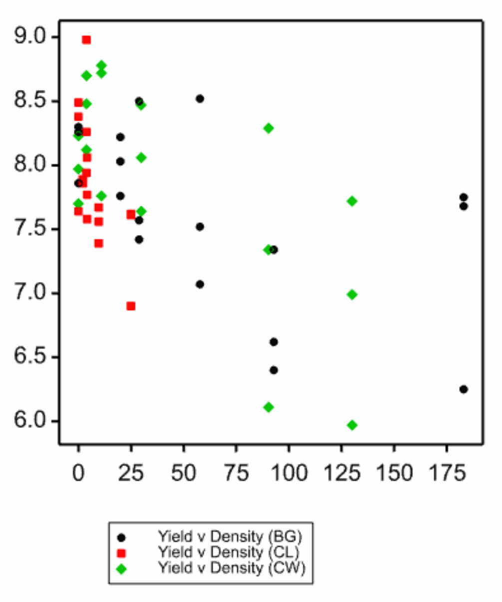

Figure S8.4.1. Plot yield against average plant density for three weed species.

It is clear that the achieved densities for cleavers (CL) are lower than those for blackgrass (BG) and chickweed (CW), as planned, but that the range of plant densities is much lower than had been intended for all three species. It also appears that yields are generally lower for higher weed densities.

We should also note that the densities reported are averages for each target value across the three blocks for each species, and that there might have been substantial variation in these values between blocks. If this were the case, we might think of using a regression approach to model yield response to actual weed plant density. However, we know that the number of seeds sown was the same across replicates with the same target value in different blocks, so we can reasonably think of the density groups as sharing the same treatment and use ANOVA to look for differences in yield between these groups within species.

Finally, we consider the control plots, with no weeds added (density = 0). There are 9 of these plots, with one allocated to each weed species within each block. We could easily argue that the control plots for each species are interchangeable, as they have the same treatment, and consider them as a single control treatment with 3 replicates per block. This would then be a factorial plus control structure of the type discussed in Section 8.5, and the analysis would follow as described there. However, for simplicity here, we keep the allocation of control plots within species and will use a simple nested structure. We therefore define a factor from the *Density* variate, with levels 1-6 for each weed species; we call this factor fDensity. The correspondence between achieved density for each weed species and levels of factor fDensity is shown in Table S8.4.1.

Table S8.4.1. Allocation of achieved plant density to levels 1-6 of factor fDensity for weed species black-grass, cleavers and chickweed.

Level of factor fDensity	Black-grass	Cleavers	Chickweed
1	0.0	0.0	0.0
2	19.9	2.1	3.8
3	28.8	3.8	10.9
4	57.7	4.1	29.8
5	92.8	9.6	90.3
6	183.0	24.9	130.0

The explanatory structure of the experiment can then be written as

Explanatory component: [1] + Weed / fDensity

The structure of the experiment is a RCBD with plots nested within blocks. The structural component of the model can therefore be written as

Structural component: Block / Plot

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

Response variate: *Yield*
Structural component: Block / Plot
Explanatory component: [1] + Weed / fDensity

The standardized residuals obtained from this model are shown in Figure S8.4.2. There is a suggestion that the variance of the residuals is larger for lower yields. This might conceivably correspond to greater variation in plant densities between replicates with higher target values, but we are unable to check this (in practice, we would consult with the experimenter before proceeding). However, the pattern is not very strong, and the histogram and Normal plots are reasonably consistent with a Normal distribution. We therefore accept these residuals as being reasonably consistent with the assumptions underlying the ANOVA, and move on to interpret the ANOVA table.

The multi-stratum ANOVA table corresponding to this nested model is shown in Table S8.4.2 below. The variance ratio for the interaction ($F^{W.fD}$ = 2.582 with 15 and 34 df, P = 0.011) gives evidence of differences in yield among levels of weed plant density within weed species. There is no evidence of differences in yield between weed species when averaged across densities (F^{W} = 1.050 with 2 and 34 df, P = 0.361).

Rather than trawl through the Weed.fDensity table of means to look for differences, we can define density factors for the individual weed species (called BG, CL and CW) and replace the amalgamated nested factor by these individual factors to test for yield differences within each weed species separately. In each case, we keep the allocation of density levels 1-6 for each weed species, and insert level 7 for plots allocated to other species. This factor allocation is shown in Table S8.4.3.

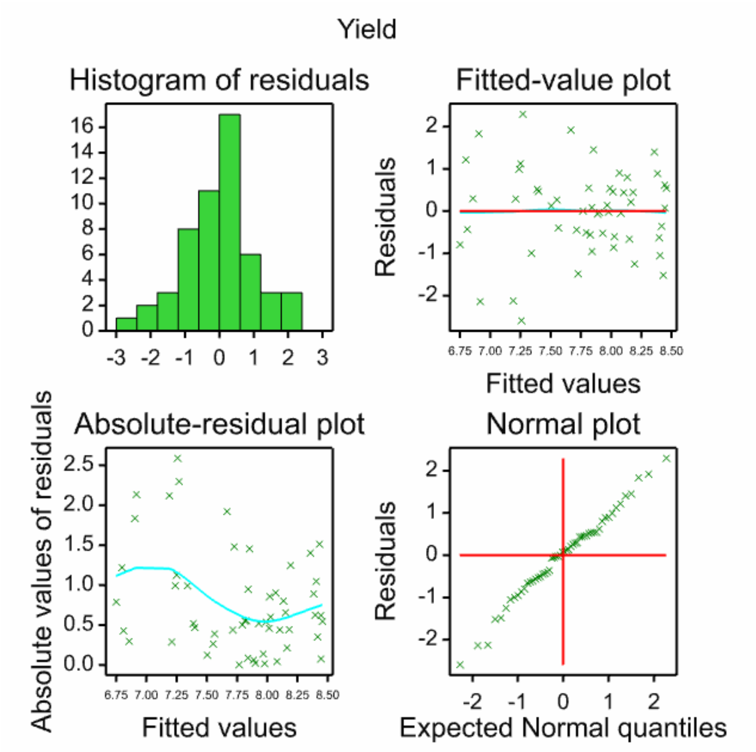

Figure S8.4.2. Residual plots for standardized residuals of plot yield from nested model.

Table S8.4.2. Multi-stratum ANOVA table for nested model for plot yields.

Source of variation	df	Sum of squares	Mean square	Variance ratio	*P*
Block stratum					
Residual	2	0.0362	0.0181	0.058	
Block.Plot stratum					
Weed	2	0.6569	0.3285	F^{W} = 1.050	0.361
Weed.fDensity	15	12.1110	0.8074	$F^{W.fD}$ = 2.582	0.011
Residual	34	10.6311	0.3127		
Total	53	23.4352			

Table S8.4.3. Allocation of weed species and achieved plant density to levels 1-7 of factors BG, CL and CW, to allow separation of nested effects between species.

Weed & fDensity levels	Factor BG level	Weed & fDensity levels	Factor CL level	Weed & fDensity levels	Factor CW level
BG & 0.00	1	CL & 0.00	1	CW & 0.00	1
BG & 19.90	2	CL & 2.10	2	CW & 3.80	2
BG & 28.80	3	CL & 3.80	3	CW & 10.90	3
BG & 57.70	4	CL & 4.10	4	CW & 29.80	4
BG & 92.80	5	CL & 9.60	5	CW & 90.30	5
BG & 183.00	6	CL & 24.90	6	CW & 130.00	6
CL or CW & any	7	BG or CW & any	7	BG or CL & any	7

The model can then be re-written in terms of these individual nested factors as

Response variate: *Yield*
Structural component: Block / Plot
Explanatory component: [1] + Weed / (BG + CL + CW)

The multi-stratum ANOVA table corresponding to this model is Table S8.4.4. It is straightforward to verify that the df and SS for the terms Weed.BC, Weed.CL and Weed.CW sum together to the quantities for Weed.fDensity in Table S8.4.2, i.e. this gives a partition of the df and SS from the previous model. The variance ratios from this ANOVA table give no evidence of differences in yield among different densities of cleavers ($F^{W.CL}$ = 1.382 with 5 and 34 df, P = 0.256). There is some evidence of differences among densities of black-grass ($F^{W.BG}$ = 2.528 with 5 and 34 df, P = 0.048) and strong evidence of differences among densities of chickweed ($F^{W.CW}$ = 3.837 with 5 and 34 df, P = 0.007). We proceed to examine tables of predicted means for black-grass and chickweed treatments, as shown in Table 8.4.5.

Table S8.4.4. Multi-stratum ANOVA table for nested model for plot yields.

Source of variation	df	Sum of squares	Mean square	Variance ratio	*P*
Block stratum					
Residual	2	0.0362	0.0181	0.058	
Block.Plot stratum					
Weed	2	0.6569	0.3285	F^{W} = 1.050	0.361
Weed.BC	5	3.9522	0.7904	$F^{W.BG}$ = 2.528	0.048
Weed.CL	5	2.1601	0.4320	$F^{W.CL}$ = 1.382	0.256
Weed.CW	5	5.9987	1.1997	$F^{W.CW}$ = 3.837	0.007
Residual	34	10.6311	0.3127		
Total	53	23.4352			

Table S.8.4.5. Predicted means of plot yield (averaged over blocks) for densities of black-grass and chickweed (plants per m^2). SED between means within each factor = 0.4566, with 14 df.

Density of black-grass	Predicted mean	Density of chickweed	Predicted mean
0	8.140	0	7.967
19.9	8.003	3.8	8.433
28.8	7.830	10.9	8.420
57.7	7.703	29.8	8.057
92.8	6.787	90.3	7.247
183.0	7.227	130.0	6.893

For comparisons within each weed species (and without adjusting for multiple tests – we might reasonably use Dunnett's test here), it appears that only the second highest density of black-grass (92.8 plants per m^2) and the highest density of chickweed (130 plants per m^2) give lower yields than their respective control treatments.

There are several other approaches we might go on to try here. As discussed above, we might amalgamate the control plots within each treatment into a single treatment and use the control plus factorial structure described in Section 8.5. This has the advantage that the greater replication of the control plots will increase the power for comparisons against it, but the disadvantage that the ANOVA variance ratios will no longer give a direct comparison of control against plant densities within weed species. Alternatively, to test the hypothesis that yield decreases as a function of weed plant density, we might use polynomial contrasts, as described in Section 8.7. Again, separating the control treatments out into a common group would make it difficult to include the zero count within the polynomial fit, and so we would probably retain the separate control treatments in this approach. We leave these other approaches as exercises for the reader.