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AGR5201
Advanced Statistical Methods

Three factorial experiment

Topic outline

1.0 Three factor experiment

- Introduction
- Main effects

1.1 Interactions in 3-factor experiment

- 1st & 2nd order interaction

1.2 Three-factor analysis of variance

- ANOVA table
- Interaction
- Example – 1st order & 2nd order interaction

Reference:
Gomez, A.G & Gomez, A.A. (1984). Statistical procedures for agricultural research. John Wiley & Sons. Page 130

1.0 Three-factor Experiments

- An experiment that deals with three factors (each factor has at least two levels)
- Design selection, treatment composition, and randomization remain the same as two factorial experiment.
- Each additional factor adds a layer of complexity to the analysis
- In a 3-factor experiment we estimate and test:
    - 3 main effects
    - 3 two-factor interactions (first order)
    - 1 three-factor interaction (second order)

1.0 Three-factor Experiments

In general…

- We have ‘a’ levels of A, ‘b’ levels of B, and ‘c’ levels of C
  - Total number of plots per replication will be a x b x c

- SSTot = $\sum(Y_{ijkl} - \bar{\bar{\bar{Y}}})^2$
  - Excel spreadsheet:
    - = DEVSQ(Range of all observations)
  - With a calculator:
    - = s2*(n-1) = σ2*n, where n = rabc

1.0 Three-factor Experiments

Table for Main Effects

- Where A1 represents the mean of all the treatments involving Factor A at level 1, averaged across all of the other factors.

	Level			
	1	2	…	f
Factor A	A_1	A_2	…	A_a
Factor B	B_1	B_2	…	B_b
Factor C	C_1	C_2	…	C_c

1.0 Three-factor Experiments

Table of Means for Treatments

Treatment	Means over Reps
$A_1B_1C_1$	T_{111}
$A_1B_1C_2$	T_{112}
$A_1B_1C...$	$T_{11..}$
$A_1B_1C_c$	T_{11c}
$A_1B_2C_1$	T_{121}
$A_1B_2C_2$	T_{122}
$A_1B_2C...$	$T_{12..}$
$A_1B_2C_c$	T_{12c}
....	
$A_aB_bC_c$	T_{abc}

Compute a mean over replications for each treatment.

Total number of treatments = a x b x c.

1.1 Interactions in 3-factor experiment

1st order interaction:
Interaction between two factors:
- A x B
- A x C
- B x C

2nd order interaction:
Interaction between all factors → **A x B x C**

Factor A	Factor B			
	1	2	…	b
1	$T_{11.}$	$T_{12.}$	…	$T_{1b.}$
2	$T_{21.}$	$T_{22.}$	…	$T_{2b.}$
…	…	…	…	…
a	$T_{a1.}$	$T_{a2.}$	…	$T_{ab.}$

Compute a table such as this for each first order interaction:

A x B   A x C   B x C

1.1 Interactions in 3-factor experiment

Linear model of 3 factorial (RCBD)

$$Y_{ijkl} = \mu + \gamma_l + \alpha_i + \beta_j + \tau_k + (\alpha\beta)_{ij} + (\alpha\tau)_{ik} + (\beta\tau)_{jk} + (\alpha\beta\tau)_{ijk} + \varepsilon_{ijkl}$$

Where,

Y_{ijkl} = observation
μ = overall mean
γ_l = the effect of the l^{th} block
α_i = the effect of the i^{th} level of factor A
β_j = the effect of the j^{th} level of factor B
τ_k = the effect of the k^{th} level of factor C

$(\alpha\beta)_{ij}$ = the ij^{th} A*B interaction effect
$(\alpha\tau)_{ik}$ = the ik^{th} A*C interaction effect
$(\beta\tau)_{jk}$ = the jk^{th} B*C interaction effect
$(\alpha\beta\tau)_{ijk}$ = the ijk^{th} A*B*C interaction effect
ε_{ijkl} = random error

1.2 Three-factor analysis of variance

ANOVA table (RCBD)

Linear model:

$$Y_{ijkl} = \mu + \gamma_l + \alpha_i + \beta_j + \tau_k + (\alpha\beta)_{ij} + (\alpha\tau)_{ik} + (\beta\tau)_{jk} + (\alpha\beta\tau)_{ijk} + \varepsilon_{ijkl}$$

Sources of variation	df	SS	MS	F
Block	r-1	SSR = $abc\sum_l\left(\bar{Y}_{...l}-\bar{\bar{Y}}\right)^2$	MSR = SSR/(r-1)	F_R = MSR/MSE
A	a-1	SSA = $rbc\sum_i\left(\bar{Y}_{i...}-\bar{\bar{Y}}\right)^2$	MSA = SSA/(a-1)	F_A = MSA/MSE
B	b-1	SSB = $rac\sum_j\left(\bar{Y}_{.j..}-\bar{\bar{Y}}\right)^2$	MSB = SSB/(b-1)	F_B = MSA/MSE
C	c-1	SSC = $rab\sum_k\left(\bar{Y}_{..k.}-\bar{\bar{Y}}\right)^2$	MSC = SSC/(c-1)	F_C = MSA/MSE

…

1.2 Three-factor analysis of variance

ANOVA table (RCBD) - cont'd

Sources of variation	df	SS	MS	F
AB	(a-1)(b-1)	SSAB= $rc\sum_{ij}\left(\bar{Y}_{ij..}-\bar{\bar{Y}}\right)^2-SSA-SSB$	MSAB = SSAB / df_{ab}	F_{AB} = MSAB/MSE
AC	(a-1)(c-1)	SSAC= $rb\sum_{ik}\left(\bar{Y}_{i.k.}-\bar{\bar{Y}}\right)^2-SSA-SSC$	MSAC = SSAC / df_{ac}	F_{AC} = MSAC/MSE
BC	(b-1)(c-1)	SSBC= $ra\sum_{jk}\left(\bar{Y}_{.jk.}-\bar{\bar{Y}}\right)^2-SSB-SSC$	MSBC = SSBC / df_{bc}	F_{BC} = MSBC/MSE
ABC	(a-1)(b-1)(c-1)	SSABC= $r\sum_{ijk}\left(\bar{Y}_{ijk.}-\bar{\bar{Y}}\right)^2-SSA-SSB-SSC$ $-SSAB-SSAC-SSBC$	MSABC = SSABC / df_{abc}	F_{ABC} = MSABC/MSE
Error	(r-1)(abc-1)	SSE= SSTot-SSR-SSA-SSB-SSC -SSAB-SSAC-SSBC-SSABC	MSE = SSE/ df_e	
Total	rabc-1	SSTot= $\sum_{ijkl}\left(Y_{ijkl}-\bar{\bar{Y}}\right)^2$		

1.2 Three-factor analysis of variance

Interpretation

The interpretation depends on the outcome of the F tests for main effects and interactions:

- If the **'3-factor (AxBxC)' interaction is significant**
  - None of the factors are acting independently
  - Summarize with 3-way table of means for each treatment combination
- If the '1st order interactions' are significant (and not the 3-factor interaction)
  - Neither of the main effects are independent
  - Summarize with 2-way table of means for significant interactions
- If only the 'main effects' are significant (and not any of the interactions)
  - Summarize significant main effects with a 1-way table of factor means

1.2 Three-factor analysis of variance

Example 1 – 1st order interaction is significant

- Study the effect of three production factors:
  - Variety (2)
    - V1, V2
  - Phosphorus fertilization (3)
    - None, 25 kg/ha, 50 kg/ha
  - Weed control (2)
    - None, Herbicide
- Using RCBD design in three blocks

1.2 Three-factor analysis of variance | 1st order interaction

3-factor ANOVA table

- RCBD – has block
- 1st order interaction
  - 2 factors interaction
- 2nd order interaction
  - 3 factors interaction
- Significance - 1st order interaction

Source	df	SS	MS	F
Block	2	270.17	135.08	5.93**
Main effects:				
Variety (V)	1	306.25	306.25	13.44**
Phosphorus (P)	2	32	16	0.70
Weed (W)	1	12.25	12.25	0.54
1st order interaction:				
V x P	2	18.67	9.33	0.41
V x W	1	283.36	283.36	12.44**
P x W	2	468.67	234.33	10.29**
2nd order interaction:				
V x P x W	2	44.22	22.11	0.97
Error	22	501.16	22.78	
Total	35	1936.75		

1.2 Three-factor analysis of variance | 1st order interaction

V x W interaction | Interpretation

The effect of herbicide depended on variety:

- The addition of herbicide reduced the yield for variety 1
- The yield of variety 2 was increased by the use of herbicide

Mean seed yield (kg/plot) from two varieties of chick-peas with and without herbicide

Variety	Weed control: None	Weed control: Herbicide
V_1	56.89	52.44
V_2	57.11	63.89

*Standard error = 1.59

1.2 Three-factor analysis of variance | 1st order interaction

P x W interaction | Interpretation

- Response to added phosphorus depended on whether or not herbicide was used
  - If no herbicide, seed yield was reduced when phosphorus was added
  - However, seed yield increased when phosphorus was added in addition to herbicide

Mean seed yield (kg/plot) of chick-peas at three levels of phosphorus fertilization with and without herbicide

Weed control	Phosphorus		
	None	25 kg/ha	50 kg/ha
None	60.00	57.83	53.17
Herbicide	52.50	58.67	63.33

*Standard error = 1.95

1.2 Three-factor analysis of variance | 1st order interaction

Figure: Line and bar graph (P x W and V x W interactions)

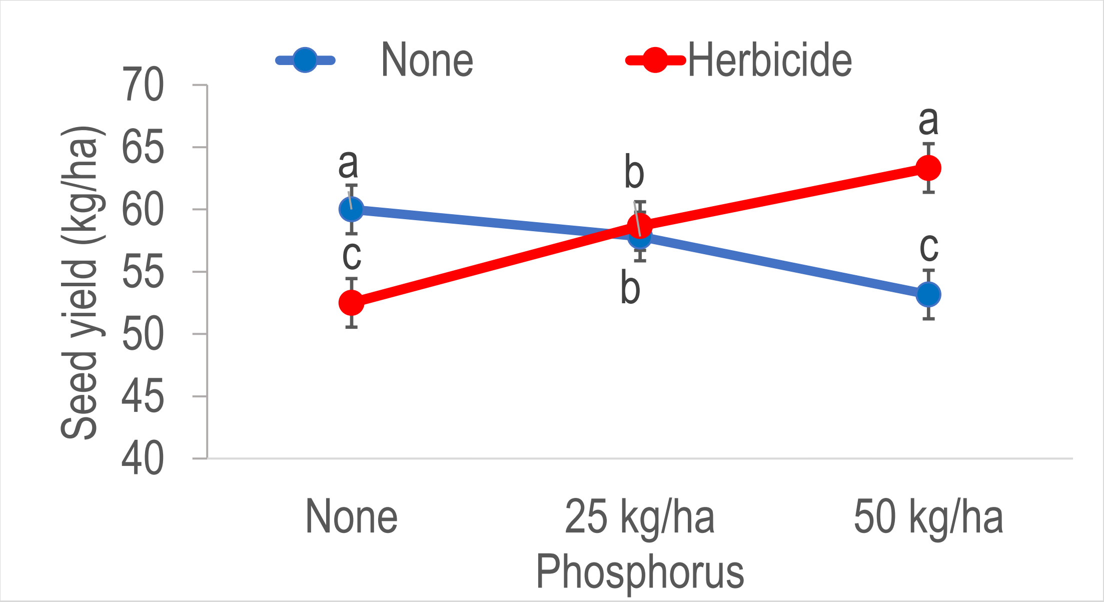


Figure 1. The interaction effect between phosphorus rate and weed control on seed yield of alfalfa (kg/ha). Within weed control, means with different letters are significantly different at P<0.05 using LSD.

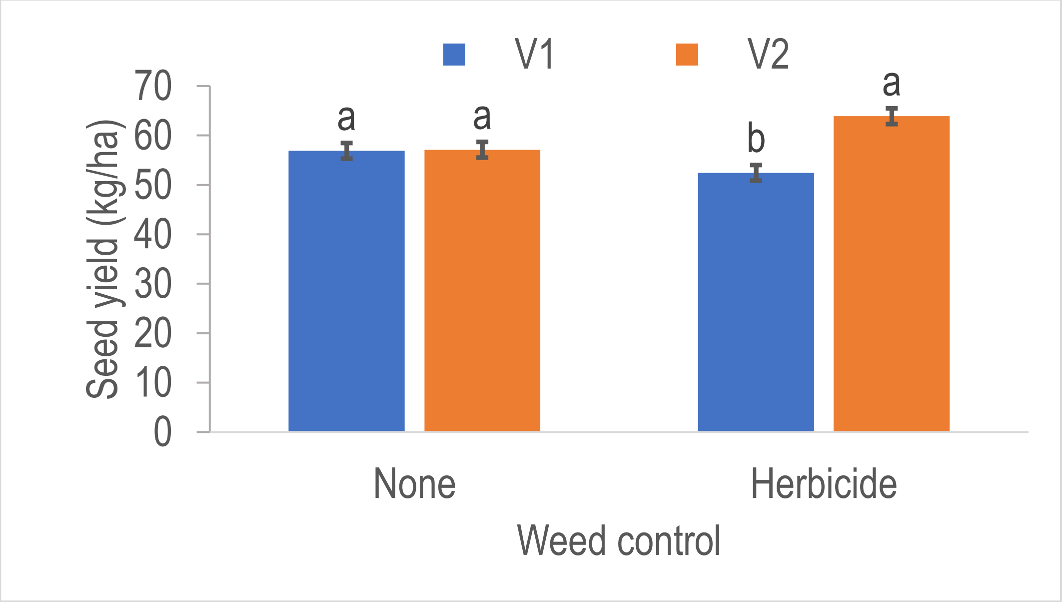


Figure 2. The interaction effect between variety and weed control on seed yield of alfalfa (kg/ha). Within weed control, means with different letters are significantly different at P<0.05 using LSD.

1.2 Three-factor analysis of variance | 2nd order interaction

Example 2 | 1st and 2nd order interaction is significant

- Objective: To study the effect of species, soil type and fungicide on seed germination.
- Design: RCBD (3 reps)
- Factor A: Legume species (alfalfa, red clover, sweet clover)
- Factor B: Soil type (Silt loam, sand, clay)
- Factor C: Fungicide (None, treated)

1.2 Three-factor analysis of variance | 2nd order interaction

Analysis of variance table

Sources of variation	df	MS	F value
Block	2	178.39	1.90
A (Species)	2	4950.06	52.60 **
B (Soil type)	2	8218.06	87.33 **
C (Fungicide)	1	1932.02	20.53 **
AxB (Species*Soil type)	4	164.61	1.75 ns
AxC (Species*Fungicide)	2	97.02	1.03 ns
BxC (Soil type* Fungicide)	2	925.57	9.84 **
AxBxC (Species*Soil type*Fungicide)	4	267.41	2.84 *
Error	34	94.10	
Total	53		

*, ** Significantly difference at P<0.05 and 0.01, respectively.
ns Not significantly difference at P<0.05

1.2 Three-factor analysis of variance | 2nd order interaction

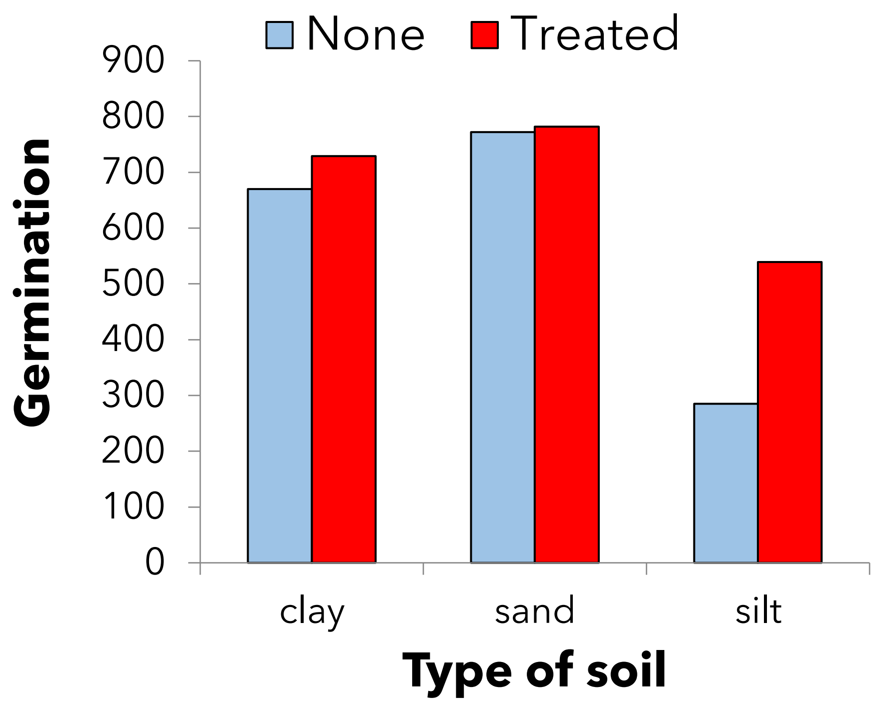
Significant interactions

- First order interaction: BxC (Soil type and fungicide)
  - The effect of fungicide is not the same for all soil type
- Second order interaction: AxBxC (Species*Soil type*Fungicide)
  - The BxC (soil type*fungicide) interaction differ with the level of A (species).

1.2 Three-factor analysis of variance | 2nd order interaction

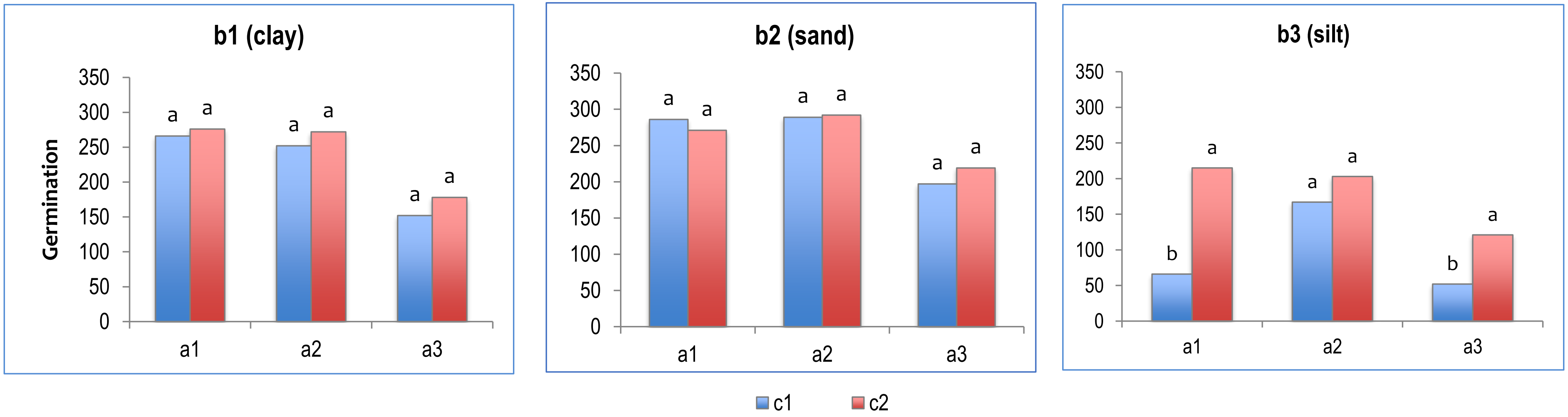
Graph of the 1st order interaction (BxC)

- The interaction between B (soil type) and C (fungicide) are the same for all level of A (species).
- The difference between fungicide c1 and c2 in soil type b1 (clay) is smaller than at soil type b3 (silt) →interaction



1.2 Three-factor analysis of variance | 2nd order interaction

Graph of the 2nd order interaction (AxBxC)



- The 2nd order interaction can be explained as AxC at each level of B (soil)
- The interaction between A and C differs at every level of B
- Interpretation should be made on the interaction effect of A and C separately on each level of B
- In this example, the AxC interaction are not significant at b1 and b2, but significant at b3 (silt soil)
- Thus, the explanation for AxC interaction can be made for b3 (silt soil) only.

1.2 Three-factor analysis of variance | 2nd order interaction

Details on the 2nd order interaction: AxC interaction at b3 (soil type = silt) only

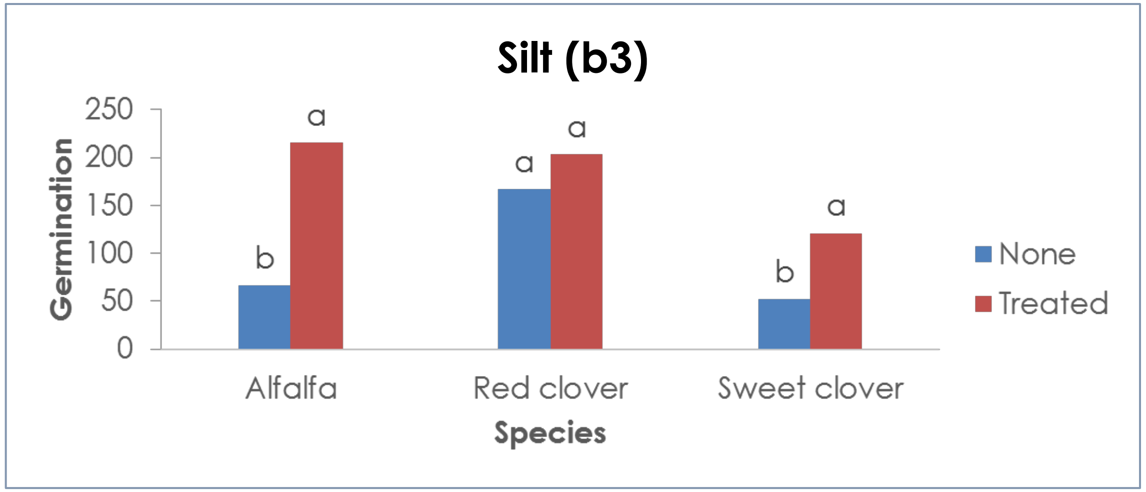


Figure 1. The interaction effect between species and fungicide treatment for silt soil type. Within species, means with different letters are significantly different at $p<0.05$ using LSD.

Interpretations:

1. For silt soil (b3), the germination of seeds depends on the species (A) and whether the seeds are treated with fungicide or not (C).
2. Fungicide treated seeds shows significantly higher number of germination compared to non treated seeds of alfalfa and sweet clover only but not for red clover.