Design of experiments

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Kourou, novembre 2010

objectives of the course

introduce Design of Experiment (DOE)

1. Basic Principles and Techniques
2. Problem formulation
3. Planning Experiments
4. Analysis data

outlines

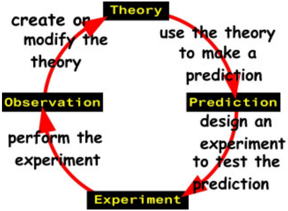
experimental method

Observation

Selection of a proportion of the population and measurement or observation of the values of the variables in question for the selected elements

Experimentation

Manipulation of the values (or levels) of one or more (independent) variables or treatments and observation of the corresponding change in the values of one or more (dependent) variables or responses



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Why experiment ?

- To determine the causes of variation in the response
- To find conditions under which the optimal response is achieved
- To compare responses at different levels of controllable variables
- To develop a model for predicting responses

some definitions

Treatments different combinations of conditions that we wish to test

Treatment Levels the relative intensities at which a treatment will be set during the experiment

Treatment Factor (or Factor) one of the controlled conditions of the experiment (these combine to form the treatments)

Experimental Unit subject on which a treatment will be applied and from which a response will be elicited also called measurement or response units

Experimental Design rule for assigning treatment levels to experimental units

Observations outcomes that will be elicited from experimental units after treatments have been applied

design of experiment

statement of

1. goals and condition of experiments
2. treatment factors and their levels
3. individuals, experimental units
4. observations and collect procedure
5. experimental design
6. data analysis $\rightarrow$ ANOVA, regression, ...

What characterizes a good experimental design ?

- It avoids systematic error: systematic error leads to bias when estimating differences in responses between (i.e., comparing) treatments
- It allows for precise estimation: achieves a relatively small random error, which in turn depends on
  - the random error in the responses
  - the number of experimental units
  - The experimental design employed
- It allows for proper estimation of error
- It has broad validity: the experimental units are a sample of the population in question

Problem formulation

- what is the biological question?
- how to answer that?
- what is already known?
- what information is missing?
- problem formulation $\rightarrow$ model of the biological system

Setting up an experiment

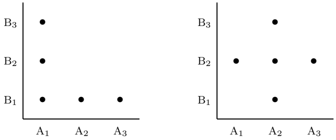
- what kind of data is needed to answer the question?
- how to collect the data?
- how much data is needed?
- biological and technical replicates
- pooling
- how to carry out the experiment (sample preparation, measurements)?

check list for planning experiment

1. Define the objectives of the experiment.
2. Identify all sources of variation, including:
   - treatment factors and their levels,
   - experimental units,
   - blocking factors, noise factors, and covariates.
3. Choose a rule for assigning the experimental units to the treatments.
4. Specify the measurements to be made, the experimental procedure, and the anticipated difficulties.
5. Run a pilot experiment.
6. Specify the model.
7. Outline the analysis.
8. Calculate the number of observations that need to be taken.
9. Review the above decisions. Revise, if necessary.

Complete factorial designs

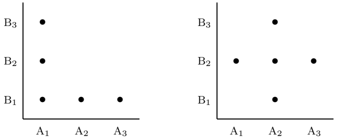
- one factor at a time



unable to assess interaction between factors

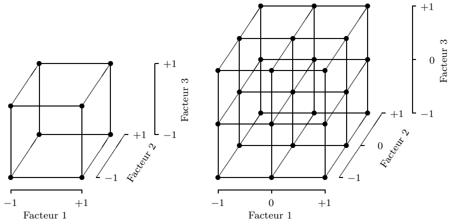
Complete factorial designs

- one factor at a time



unable to assess interaction between factors

- complete factorial designs (2^3 and 3^3)

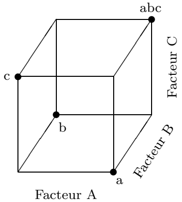


able to assess interaction between factors

need high number of treatments

Fractionnal factorial designs

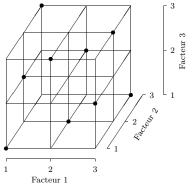
- Case $2^3 \rightarrow 2^{3-1}$
  - 3 factors with 2 levels: A ($\bar{a}$, a), B ($\bar{b}$, b) and C ($\bar{c}$, c)
  - 4 treatments: $a\bar{b}\bar{c}$, $\bar{a}b\bar{c}$, $\bar{a}\bar{b}c$ and abc



  - (A,BC), (B,AC) and (C,AB) are aliases
  - able to estimate principal effects if interactions are nulls

Fractionnal factorial designs

- Case $3^3 \to 3^{3-1}$
  - 3 factors with 3 levels: 1 $(1, 2, 3)$, 2 $(1, 2, 3)$ and 3 $(1, 2, 3)$
  - 9 treatments: 111 , 122 , 133 , 213 , 221 , 232 , 312 , 323 , 331



  - (1,23), (2,13) and (3,12) are aliases
  - able to estimate principal effects if interactions are nulls

Resolution of fractionnal factorial designs

levels of resolution

- III able to estimate principal effects if interactions factors are nulls
- IV able to estimate principal effects if interactions between three or more factors are nulls
- V able to estimate principal effects and interactions between two factors if interactions between three or more factors are nulls

case of 2^k designs

Nb. factors	Nb. tot. trait	Nb. trait min III	IV	V
3	8	4	8	8
4	16	8	8	16
5	32	8	16	16
6	64	8	16	32
7	128	8	16	64
8	256	16	16	64
9	512	16	32	128
10	1.024	16	32	128

Experimental unit

- size: the smaller the better while keeping a meaning
- edge: avoid interferences

1	1	1	1	1	2	2	2	2	2
1	**1**	**1**	**1**	1	2	**2**	**2**	**2**	2
1	**1**	**1**	**1**	1	2	**2**	**2**	**2**	2
1	1	1	1	1	2	2	2	2	2
4	4	4	4	4	3	3	3	3	3
4	**4**	**4**	**4**	4	3	**3**	**3**	**3**	3
4	**4**	**4**	**4**	4	3	**3**	**3**	**3**	3
4	4	4	4	4	3	3	3	3	3

0	0	0	0	0	0	0	0	0
0	**1**	**1**	**1**	0	**2**	**2**	**2**	0
0	**1**	**1**	**1**	0	**2**	**2**	**2**	0
0	0	0	0	0	0	0	0	0
0	**4**	**4**	**4**	0	**3**	**3**	**3**	0
0	**4**	**4**	**4**	0	**3**	**3**	**3**	0
0	0	0	0	0	0	0	0	0

- path: close to the edge
- shape: square to reduce edge effects, but frame could be good in case of heterogeneity
- number of repetition: ensure the viability of the experiment
  - mean estimation: $n \approx 4cv^2/d_r^2$ with *cv* coefficient of variation and d_r maximum relative error
  - two means comparison: $n \approx 4cv^2/\delta_r^2$ with δ_r inter mean distance

Completely Randomized Designs

goal

avoid fluctuation from uncontrolled factors though time and space

principle

- randomly affect treatment on experimental units:

4 (1)	5 (2)	4 (3)	4 (4)
3 (5)	2 (6)	2 (7)	4 (8)
1 (9)	5 (10)	5 (11)	3 (12)
1 (13)	2 (14)	2 (15)	3 (16)
3 (17)	1 (18)	5 (19)	1 (20)

- model: Response = constant + effect of treatment + error

+/-

- +: very simple to implement
- -: may lead to abnormalities due to treatments concentration and heterogeneity

Randomized Blocks

goal

address fluctuation from uncontrolled factors by blocking homogeneous experimental units

principle

- split experimental units into block
- randomly affect each treatment on experimental units into each block:

3	4	4	6
6	3	5	3
1	5	2	1
2	1	7	5
7	7	3	2
4	2	1	4
5	6	6	7
Bloc 1	Bloc 2	Bloc 3	Bloc 4

Gradient →

- model: Response = constant + effect of block + effect of treatment + error

Randomized Blocks: advantages

- very simple to implement
- more efficient than completely randomized design:
  - experiment with p blocks and q treatments
  - SSE_b sum of squares relatives to blocks
  - SSE_{tb} sum of squares relatives to interaction treatments-blocks
  - $MSE_{tb} = SSE_{tb}/[(p-1)(q-1)]$
  - $MSE_r = (SSE_b + SSE_{tb})/[(q-1)+(p-1)(q-1)]$
  - relative efficiency approximate by

$$\frac{MSE_r}{MSE_{tb}} = (p-1)(\frac{SSE_b}{SSE_{tb}} + 1)/p.$$

- the higher SSE_b the more efficient is the block design

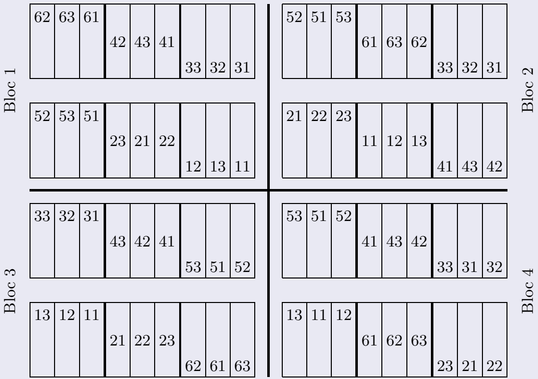
Split-plot Designs

goal

study differently the effect of each factors

principle

case of 2 factors (6 and 3 levels) and 4 blocks:



Split-plot Designs

+

- allow to consider larger experimental units for first factors
- better accuracy for factor on the small experimental units (more repetitions)
- better accuracy for interaction
- allow to introduce a new factor during the experiment

-

- lost in accuracy for factor on the larger experimental units (less repetitions)
- different number of degree of freedom

Cross-over Designs

- goal: control variability of experimental material
- principle
  - latin square

3	1	2	4
1	4	3	2
4	2	1	3
2	3	4	1

  - cross-over

4	3	3	2	1	1	2	4
2	1	4	3	4	3	1	2
3	2	1	4	3	2	4	1
1	4	2	1	2	4	3	3

- +/-
  - more efficient than random blocks because of double control
  - low degree of freedom for residuals variability
  - number of treatments = number of repetitions

fractionnal factorial experiment

- principle
  - number total of treatments>number of experimental unit per block
  - incomplete blocks completed by repetitions
- example
  - 2^3 design with 4 experimental units per block

ac	bc	ab	(1)
b	a	abc	c
a	abc	c	b
bc	(1)	ac	ab
c	a	b	abc
bc	ab	(1)	ac

  - 2^2 design with 2 experimental units per block

b	(1)	b	ab	ab	(1)	a	(1)	(1)	ab	a	ab
a	ab	(1)	a	a	b	ab	b	a	b	(1)	b

- +/-
  - adapted to situations that need small blocks
  - increase accuracy for a large number of treatments
  - confounding effects
  - data analysis is complex

Completely Randomized Designs: Charcoal beech

goal

study the influence of the size and the moisture of the piece of wood on coal yield

factors levels

- wood cube sizes: 2, 4 and 8 cm for edge
- moisture: 0%, 10%, 20% and 40%

Experimental Design

- 36 experimental units
- 12 treatments (3×4)
- 3 repetitions

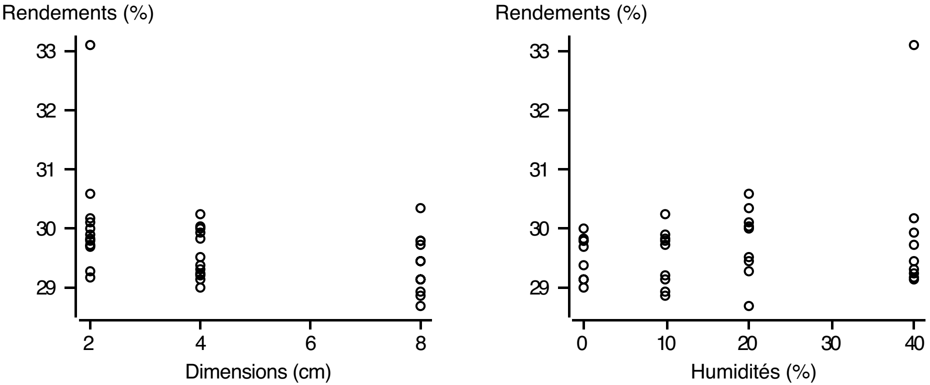
1^{st} representation of the data

size (cm)	moisture 0	10	20	40
2	30,00	29,82	29,27	33,11
	29,67	29,71	30,11	30,18
	29,78	29,87	30,58	29,16
4	29,38	29,11	29,98	29,31
	28,98	29,18	30,02	29,22
	29,82	30,22	29,49	29,93
8	29,11	28,93	28,67	29,13
	29,78	29,78	29,44	29,42
	29,11	28,84	30,33	29,73

2^{nd} representation of the data

s	m	k	y	s	m	k	y	s	m	k	y
2	0	1	30,00	4	0	1	29,38	8	0	1	29,11
2	0	2	29,6	4	0	2	28,98	8	0	2	29,78
2	0	3	29,78	4	0	3	29,82	8	0	3	29,11
2	10	1	29,82	4	10	1	29,11	8	10	1	28,93
2	10	2	29,71	4	10	2	29,18	8	10	2	29,78
2	10	3	29,87	4	10	3	30,22	8	10	3	28,84
2	20	1	29,27	4	20	1	29,98	8	20	1	28,67
2	20	2	30,11	4	20	2	30,02	8	20	2	29,44
2	20	3	30,58	4	20	3	29,49	8	20	3	30,33
2	40	1	33,11	4	40	1	29,31	8	40	1	29,13
2	40	2	30,18	4	40	2	29,22	8	40	2	29,42
2	40	3	29,16	4	40	3	29,93	8	40	3	29,73

preliminary graphical data exploration



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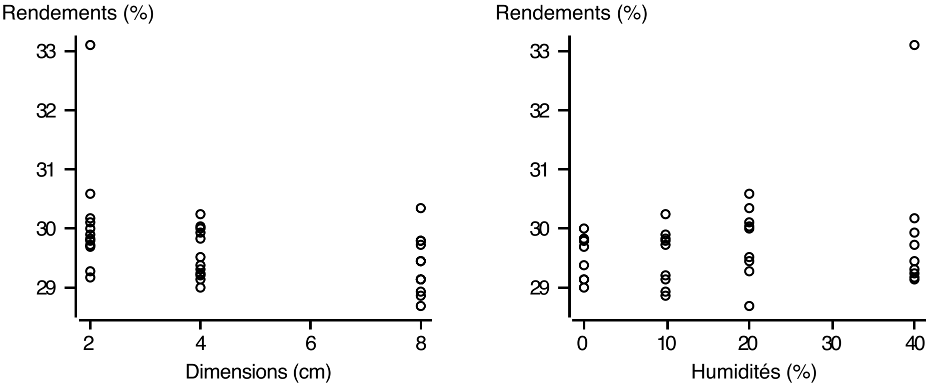


- input error for point 33.11 ?
- ANOVA . . .

Complete Block Design: Paracou

question

Can we possibly increase the production of timber within the managed areas without exhausting the resources ?

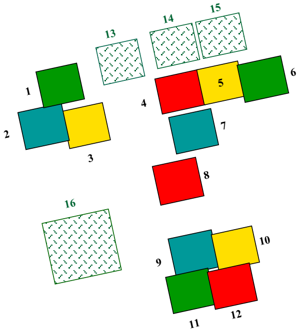
factor levels

- logging intensity: 3 increasing levels + control

Experimental Design

- 12 experimental units
- 4 treatments
- 3 blocks

the Paracou experimental station



- Control plots
- Treatment 1 : Timber harvesting
- Treatment 2 : Timber harvesting and poisoning of non commercial species
- Treatment 3 : Timber and fire wood harvesting + poisoning of non commercial species
- Untreated Plots

Treatments description

	N/ha	m^2/ha	m^3/ha
control	620	31	360
T1	10	3	50
T2	10 + 30	3 + 7	50 + 80
T3	30 + 15	6 + 3,5	80 + 50

fertilizers on wheat in Rwanda

goal

study the influence of fertilizers on wheat yield

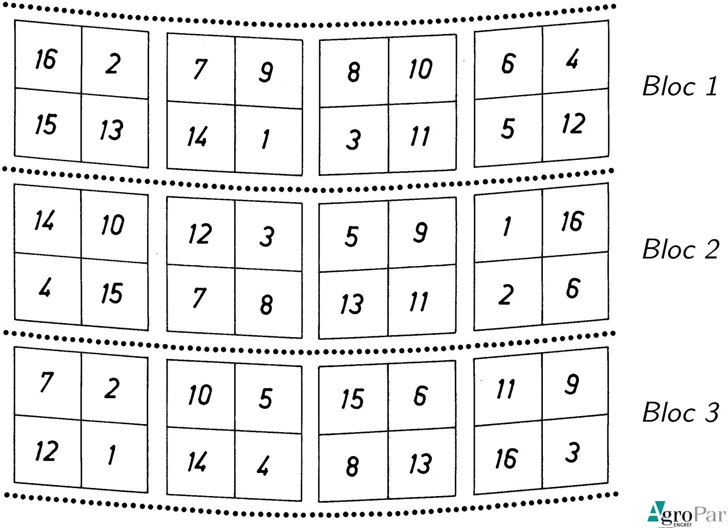
factors levels

- azote (N): 0 and 100 kg/ha
- potassium (K_2O): 0 and 200 kg/ha
- phosphore (P_2O_5): 0 , 100 , 200 and 300 kg/ha
- calcium (Ca): 1, 4.5 and 8 kg/ha

Experimental Design

- 48 experimental units: weight of wheat (kg) for 18 m^2 (centred in the 25 m^2 plot)
- 16 treatments
- 3 blocks

fertilizers on wheat in Rwanda: Experimental Design



fertilizers on wheat in Rwanda: Experimental Design

exp. unit	N	K_2O	P_2O_5	Ca
1				
2				1.000
3				4.500
4				8.000
5	100	200		1.000
6	100	200		4.500
7	100	200		8.000
8	100	200	100	1.000
9	100	200	100	4.500
10	100	200	100	8.000
11	100	200	200	1.000
12	100	200	200	4.500
13	100	200	200	8.000
14	100	200	300	1.000
15	100	200	300	4.500
16	100	200	300	8.000

fertilizers on wheat in Rwanda: anti-erosion hedges

picture from P. Dagnelie

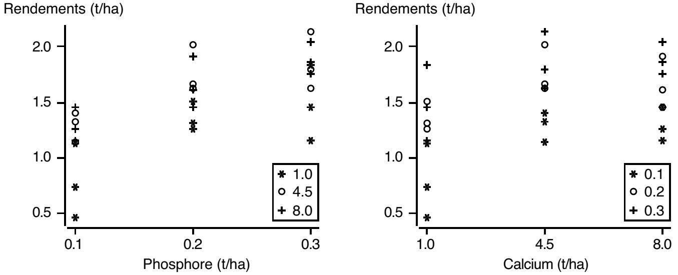
fertilizers on wheat in Rwanda: wheat yield

exp.unit	blocks			means	
	1	2	3	(kg/p)	(t/ha)
1	0,00	0,04	0,06	0,03	0,02
2	0,14	0,22	0,35	0,24	0,13
3	0,42	0,45	0,44	0,44	0,24
4	0,44	0,28	0,84	0,52	0,29
5	0,28	0,49	0,33	0,37	0,20
6	1,09	1,17	0,84	1,03	0,57
7	0,79	0,94	0,82	0,85	0,47
8	1,30	0,80	2,01	1,37	0,76
9	2,05	2,37	2,52	2,31	1,29
10	2,07	2,60	2,25	2,31	1,28
11		2,36	2,71	(2,54)	(1,41)
12	2,99	2,92	3,63	3,18	1,77
13	2,62	2,89	3,43	2,98	1,66
14	2,61	2,06	3,29	2,65	1,47
15	3,22	2,93	3,85	3,33	1,85
16	3,15	3,35	3,67	3,39	1,88

fertilizers on wheat in Rwanda: a plot with weak yield

picture from P. Dagnelie

preliminary graphical data exploration



ANOVA for exp. units 8 to 16

	Df	mse	F	P	
Phosphore	2	6,2949	3,1474	39,9 ***	0,0000
Calcium	2	3,5022	1,7511	22,2 ***	0,0000
Interaction	4	0,1525	0,0381	0,48	0,75
Blocs	2	1,9191	0,9596		
residuals	15	1,1820	0,0788		
totals	25	13,0507			

Split Block criss cross: improvement of beef cattle

goal

compare different mixtures of forage associated with two doses of nitrogen fertilizer

factor levels

- oats and vetch proportions: 50-50 and 25-75
- oats variety: A,B and C
- dose of fertilizer: 30 and 60 N

Experimental Design

- 32 experimental units, plots 8×20 m
- 16 treatments (8 mixtures $\times$ 2 doses of fertilizer)
- 2 blocks

experimental design

forage mixture design

Mixture	oats			vetch
	A	B	C	
1	50			50
2	25			75
3		50		50
4		25		75
5			50	50
6			25	75
7	25	25		50
8	12,5	12,5		75

DOE and yield t/ha

62 5,79	42 8,67	12 7,97	82 7,61	52 8,69	72 10,61	22 7,72	32 8,78	71 6,68	51 9,61	81 3,55	11 4,83	31 4,32	41 7,25	61 5,30	21 3,89
61 6,03	41 7,16	11 4,92	81 4,63	51 7,70	71 6,36	21 6,14	31 5,79	72 5,52	52 5,81	82 5,07	12 8,16	32 9,12	42 8,85	62 5,57	22 6,19

Bloc 1 Bloc 2

Conclusion

- always plan an experiment
- write your DOE:
  1. objectives
  2. experiment conditions
  3. factors
  4. treatments
  5. experimental units
  6. observations
  7. experimental design
  8. framework of data analysis

Some references

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