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Effect of not randomizing the first replicate

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Content

- The problem
- About data
- What we did
- Results and outlook

Prinziples of experimental design

- Randomization
- Replication
- Blocking

(Fisher, 1925)

+However, in order to draw valid conclusions from the experiment, we need to randomize in a valid way. (Bailey)

Randomization

- However, in order to draw valid conclusions from the experiment, we need to randomize in a valid way.

(Bailey, 2025)

Randomization

- Unbiased estimates of means and error
- Protection against different forms of spatial trend
- Prerequisite for obtaining valid statistical inferences

(Cox 2009; Piepho et al., 2013)

The problem

	6	11	5	2	9	1	12	10	3	8	4	7	Replicate 4
	8	4	10	12	3	7	6	2	11	1	9	5	Replicate 3
	9	12	7	11	8	10	4	1	5	2	6	3	Replicate 2
	1	2	3	4	5	6	7	8	9	10	11	12	Replicate 1

Understanding the problem

- Use for demonstration purpuses
- Schuster and Lochow
- „It could have occured randomly, too“

(van Santen and West, 2012)

What does the literature tells us?

- Assume known heterogenity in the field
- Assume that treatments were allocated along the gradient (e.g. best treatment on best plots)

➔ biased results

(van Santen and West, 2012)

What does the literature tells us?

- Assume gradient in the field
- Assume that treatments were replicated in pseudo-replicates

➔ biased results

(b) Systematic design

A	A	A	A
B	B	B	B
C	C	C	C
D	D	D	D
E	E	E	E

(Piepho, 2013)

Not randomizing 1st replicate – is it a problem?

- No papers specific on this topic

Not randomizing 1st replicate – is it a problem?

6	11	5	2	9	1	12	10	3	8	4	7	Replicate 4
8	4	10	12	3	7	6	2	11	1	9	5	Replicate 3
9	12	7	11	8	10	4	1	5	2	6	3	Replicate 2
1	2	3	4	5	6	7	8	9	10	11	12	Replicate 1

Not randomizing 1st replicate – is it a problem?

- No, in single experiments with randomized treatment list
- We further assume that estimated means are unbiased in series of experiment if no neighboring effects exists

But it is a problem! Isn´t it?

6	11	5	2	9	1	12	10	3	8	4	7	Replicate 4
8	4	10	12	3	7	6	2	11	1	9	5	Replicate 3
9	12	7	11	8	10	4	1	5	2	6	3	Replicate 2
1	2	3	4	5	6	7	8	9	10	11	12	Replicate 1

How can I prove that it is a problem?

6	11	5	2	9	1	12	10	3	8	4	7	Replicate 4
8	4	10	12	3	7	6	2	11	1	9	5	Replicate 3
9	12	7	11	8	10	4	1	5	2	6	3	Replicate 2
1	2	3	4	5	6	7	8	9	10	11	12	Replicate 1

Approach:

- 1st step: Need data
- 2nd step: Estimate empirical standard errors for all treatment differences ($s.e.d._{emp}$)
- 3rd step: Plot $s.e.d._{emp}$ against the distance in the first replicate (or the average distance across all four replicates)

1st step: Need data

- Historical winter wheat cultivar evaluation trials in Freising
- Data 2014 to 2024

The field plan (in each year)

6	11	5	2	9	1	12	10	3	8	4	7	Replicate 4
8	4	10	12	3	7	6	2	11	1	9	5	Replicate 3
9	12	7	11	8	10	4	1	5	2	6	3	Replicate 2
1	2	3	4	5	6	7	8	9	10	11	12	Replicate 1

1st step: Need data

- Historical winter wheat cultivar evaluation trials in Freising
- Data 2014 to 2024
- Same field plan in all years, varying fields across years
- Kernel harvest (no neighbor effects)
- 12 cultivars, 4 replicates

ID	Cultivar	Year of release
1	Rimpaus	1888
2	Tassilo	1930
3	Heine IV	1940
4	Heine VII	1950
5	Jubilar	1961
6	Diplomat	1966
7	Komoran	1973
8	Kanzler	1980
9	Bussard	1990
10	Akteur	2003
11	Elixer	2012
12	Hy Hyvega	2020

1st step: Need data

- Historical winter wheat cultivar evaluation trials in Freising
- Data 2014 to 2024
- Same field plan in all years, varying fields across years
- Kernel harvest (no neighbor effects)
- 12 cultivars, 4 replicates
- Systematic treatment list (sorted by release date)

Perfect data?

No,

- Some cultivars were changed in time
- In early years 11 cultivars, in recent years 12
- Some observations were missing
- In one of four replicates another (second) factor was added, that is also varying across years
- Sowing errors occured in replicate 1 (year 2016)
- …

but it is nearly balanced for cultivars 1 to 9

2nd step: Calculate empirical standard error of cultivar differences (s.e.d.$_{emp}$)

- Calculate pairwise differences of cultivars in each replicate and year (assuming RCBD)
- Substract average cultivar pair-by-year difference

- Calculate standard error from these centered differences for each pair of cultivars

2nd step: Calculate empirical standard error of cultivar differences ($s.e.d._{emp}$)

- Calculate pairwise differences of cultivars in each replicate and year (assuming RCBD) → removes replicate and year effects
- Substract average cultivar pair-by-year difference → removes cultivar-by-year effects
- Calculate standard error of these centered differences for each pair of cultivars

2nd step: Calculate empirical standard error of cultivar differences (s.e.d.$_{emp}$)

- $y_i = \mu + \tau_i + a_j + r_{jk} + (\tau a)_{ij} + e_{ijk}$

μ intercept

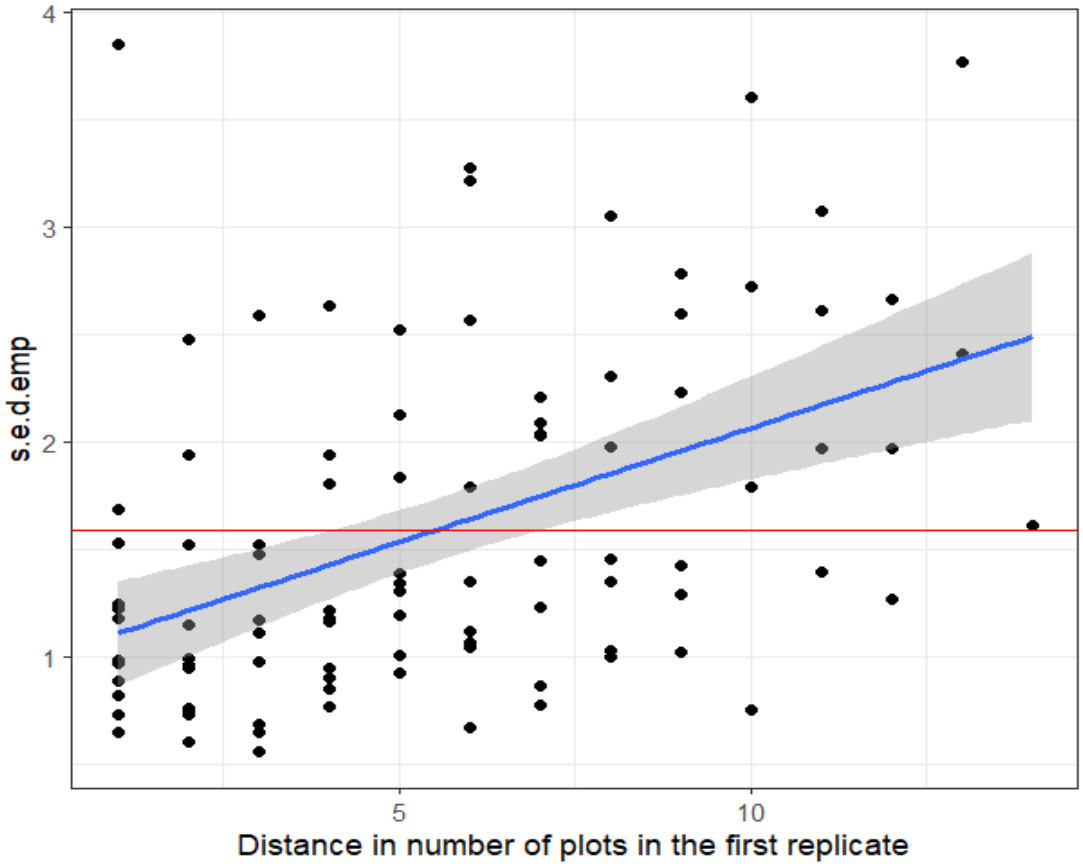
τ_i fixed effect of cultivar *i*

a_j fixed effect of year *j*

r_{jk} random effect of replicate *k* in year *j*

$(\tau a)_{ij}$ fixed effect of cultivar *i* in year *j*

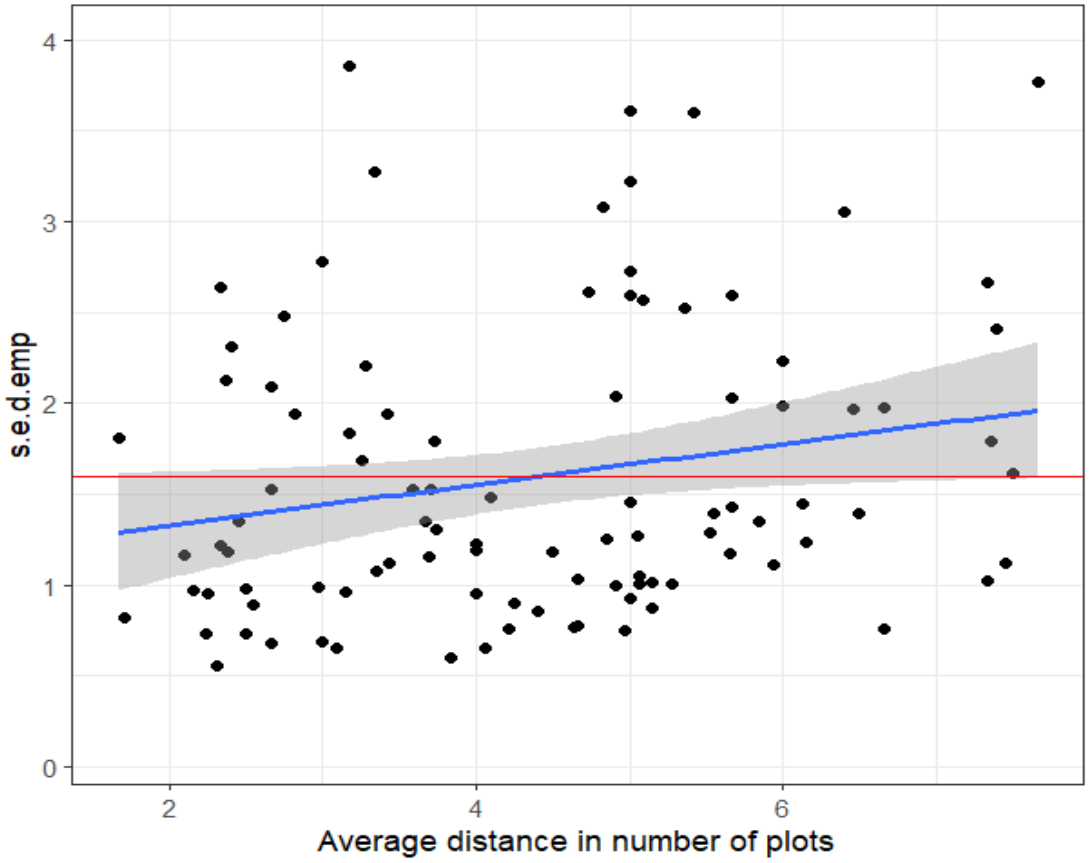
3rd step: Plot standard error against distance of cultivars in first replicate / average distance



$p < 0.0001$

$R^2 = 0.20$

$y = 1 + 0.11 * distance$



$p < 0.0347$

$R^2 = 0.05$

$y = 1.1 + 0.11 * distance$

Results and Outlook (1/2)

- Over-estimates precision of distant cultivar pairs
- Under-estimate precision of neighboring cultivar pairs

Results and Outlook (2/2)

- Are there other options to show that not randomizing the first replicate is a bad idea?

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Thank you

for your attention!

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