Bavarian State Research Center
for Agriculture

Quality over quantity? – The optimal allocation of quality samples in Bavarian cultivar evaluation trials in perennial ryegrass

A.-K. Gorn, J. Hartung, S. Hartmann, H.-P. Piepho

Fig. 1: Perennial ryegrass in plot trial, S. Hartmann, LfL

Anne-Katrin Gorn, 13th Working Seminar on Statistical Methods in Variety Testing,
COBORU, 7th - 12th September 2025

1. Perennial ryegrass

- Most important grass in grassland
- Cultivar evaluation trials for recommendation of most suitable cultivar for a specific region (Graf et al. 2009)

- Selection criterion predominantly yield
- Quality aspects are also of importance

Fig. 1: Perennial ryegrass in plot trial, S. Hartmann, LfL

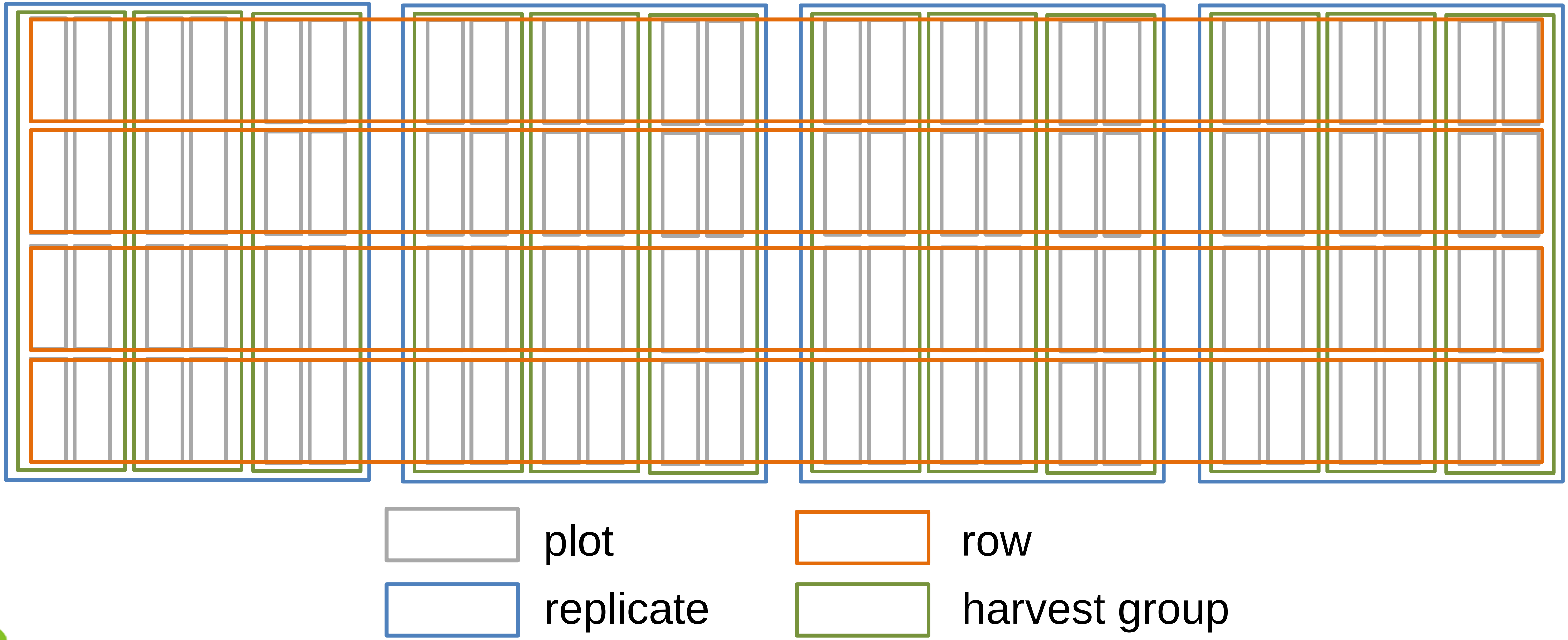
2. Evaluation trials in perennial ryegrass

Site	Trial	2016	2017	2018	2019	2020	2021	2022	2023
Osterseeon	1	Sowing year	Harvest year 1	Harvest year 2	Harvest year 3				

Trial

2. Evaluation trials in perennial ryegrass

Trial design



2. Evaluation trials in perennial ryegrass

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- Staggered starts (Loughin 2006)
- Effects for **sowing year, calendar year** and **harvest year** (Piepho and Eckl 2014)

2. Evaluation trials in perennial ryegrass

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Cycle

2. Evaluation trials in perennial ryegrass

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Series = Multi-environment trial

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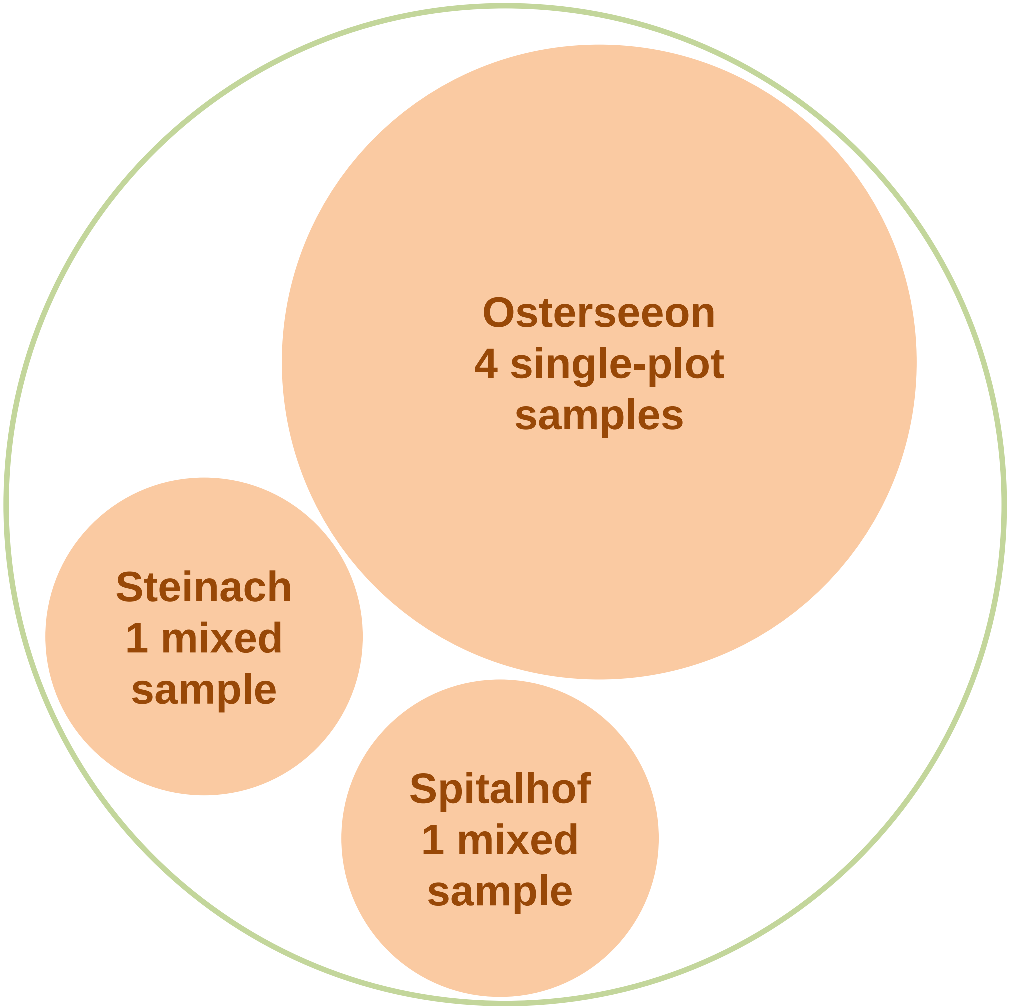
3. Problem

- 3 sites each with 4 replicates per cultivar and cut
- Number of quality samples sent to the laboratory annually is limited

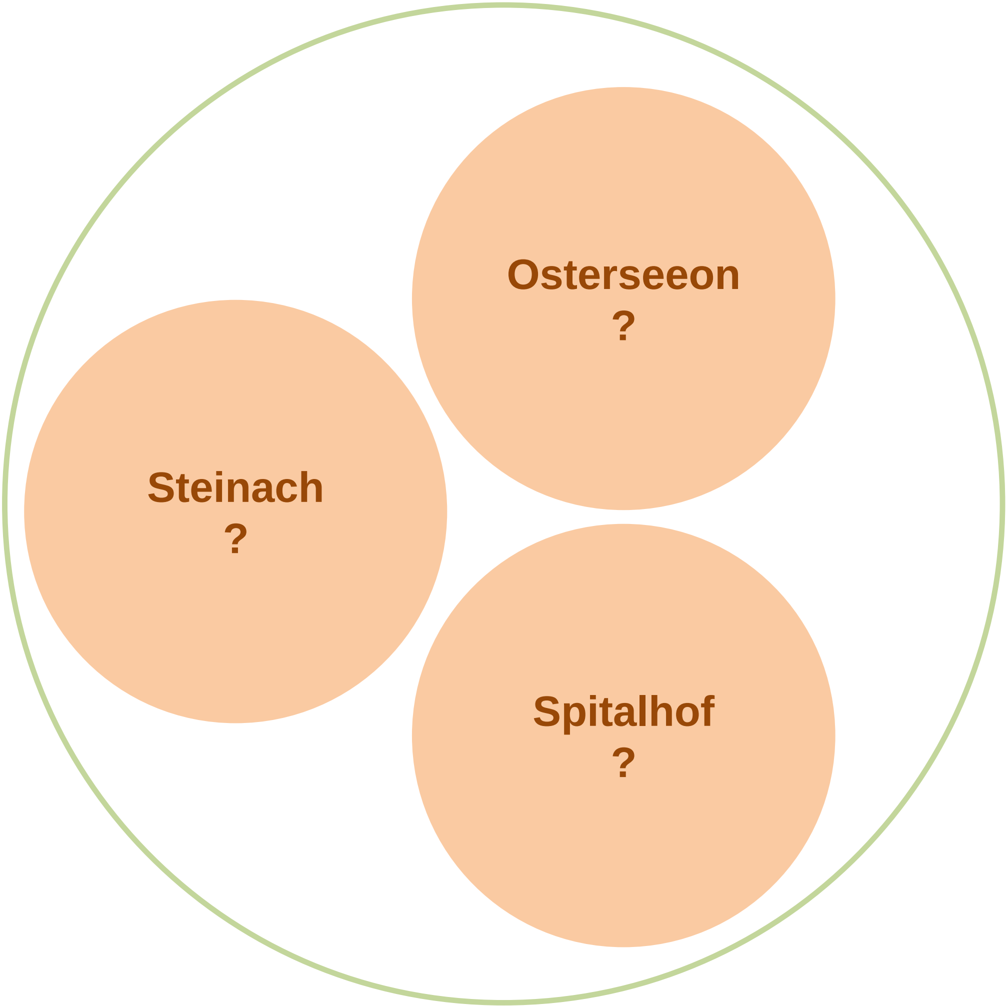
→ Single-plot samples partly mixed across the 4 replicates per cultivar and cut

→ Allocation of mixed samles good for evaluation of cultivars?



= 6 samples per cultivar and cut

4. Research question



≤ 6 samples per cultivar and cut

5. Methods - overview

1. Estimation of variance components

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2. Simulation of alternative designs

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3. Comparison of designs

5.1. Estimation of variance components

1. Estimation of variance components

2. Simulation of alternative designs

3. Comparison of designs

- Linear mixed model
- Autocorrelation due to perenniality
- Differentiation of the effects of **sowing year, calendar year and harvest year** (Piepho and Eckl 2014)

Which year effects are estimable?

- **Separate year effects?**
- A reduced number of parameters for **environments?**

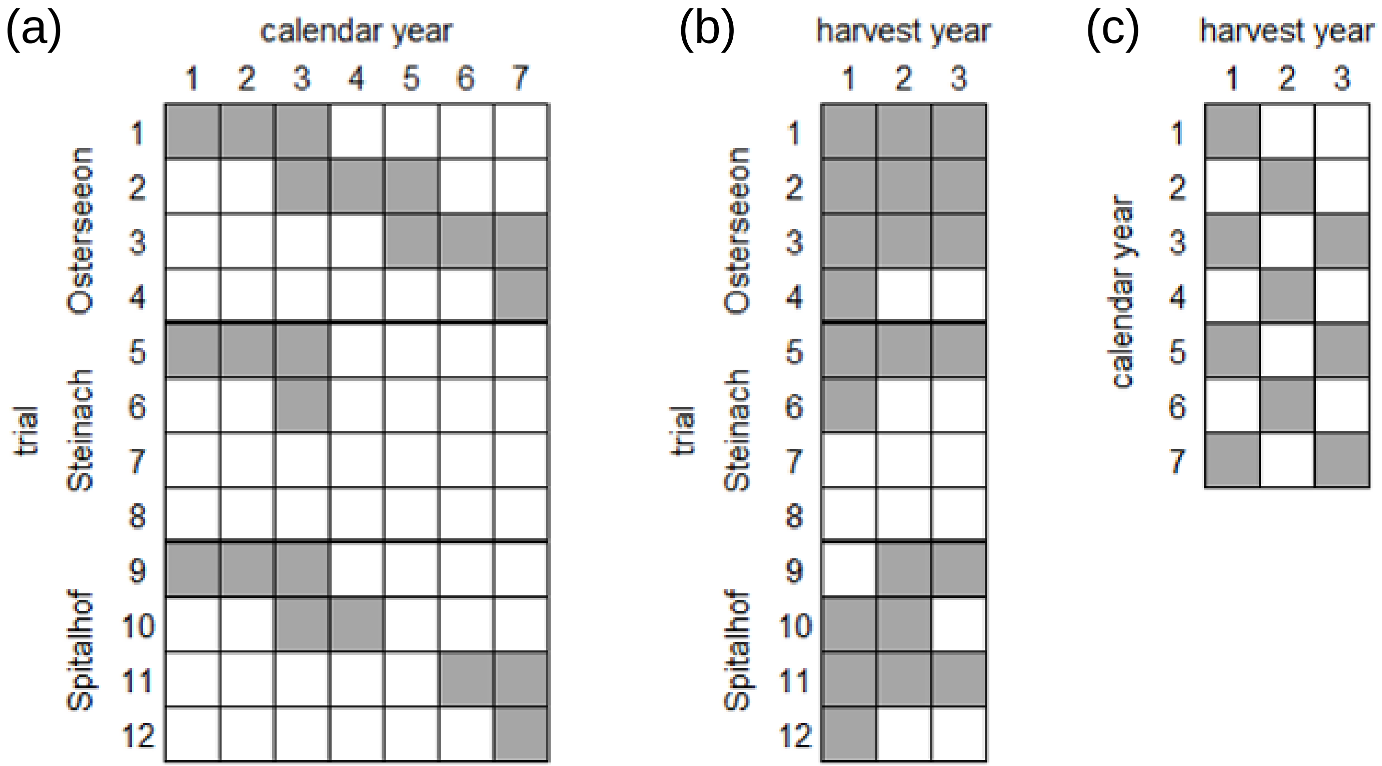
A strategy

5.1.1. A strategy for year effects in perennial crops

Estimability of year effects ?

5.1.1. A strategy for year effects in perennial crops

- For estimating **main effects**, connected data is needed (Searle 1987)



5.1.1. A strategy for year effects in perennial crops

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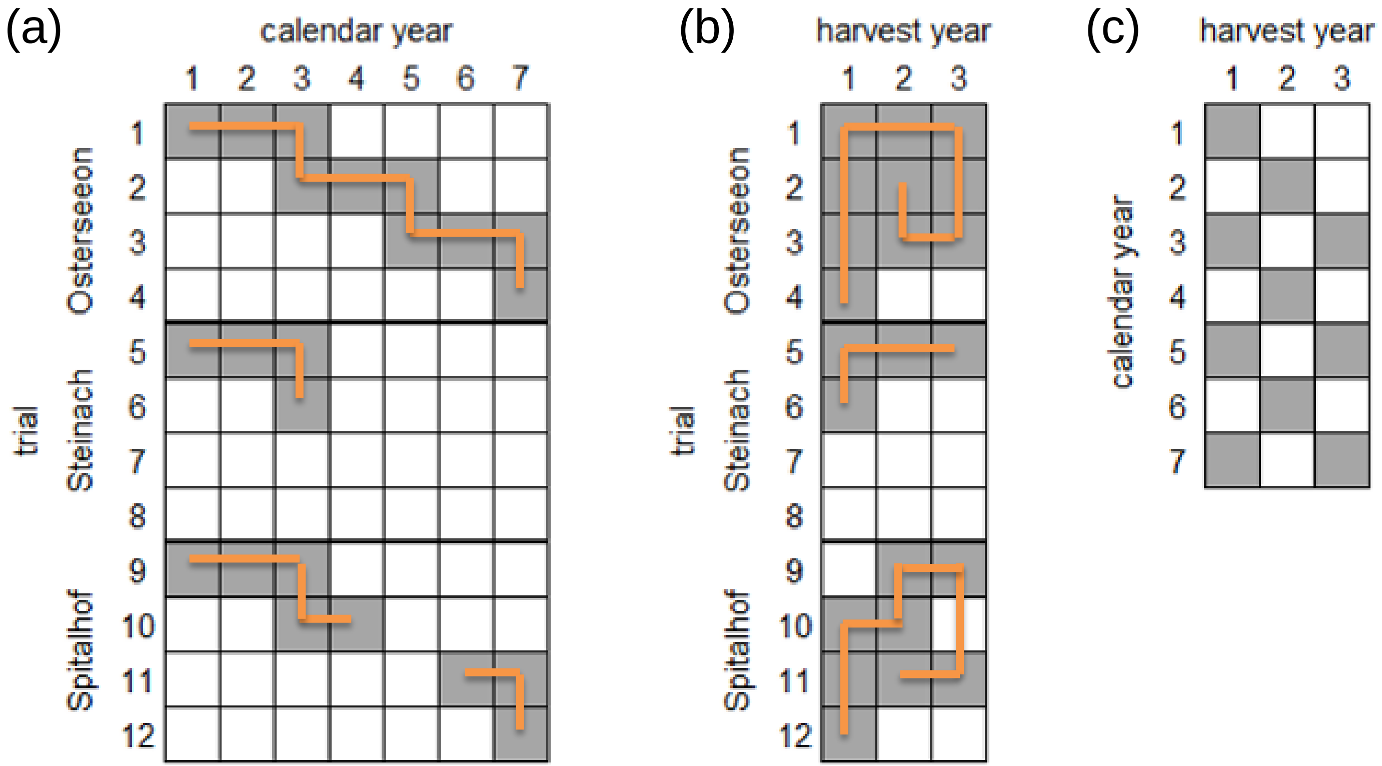
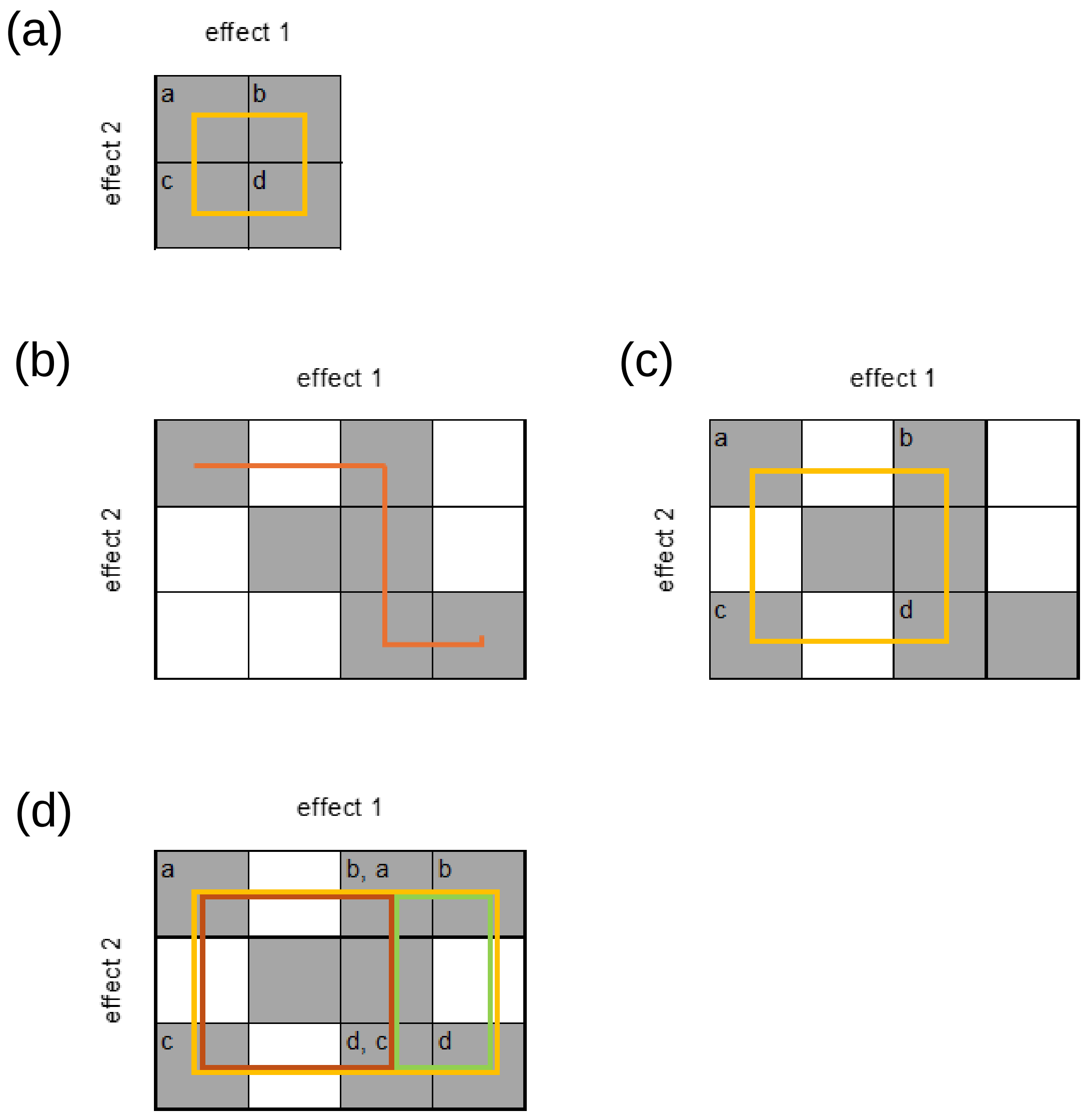


5.1.1. A strategy for year effects in perennial crops

- Tetrads necessary for estimating **two-way interactions** (Bradu and Gabriel 1974)
- Tetrad: $(a - b) - (c - d)$



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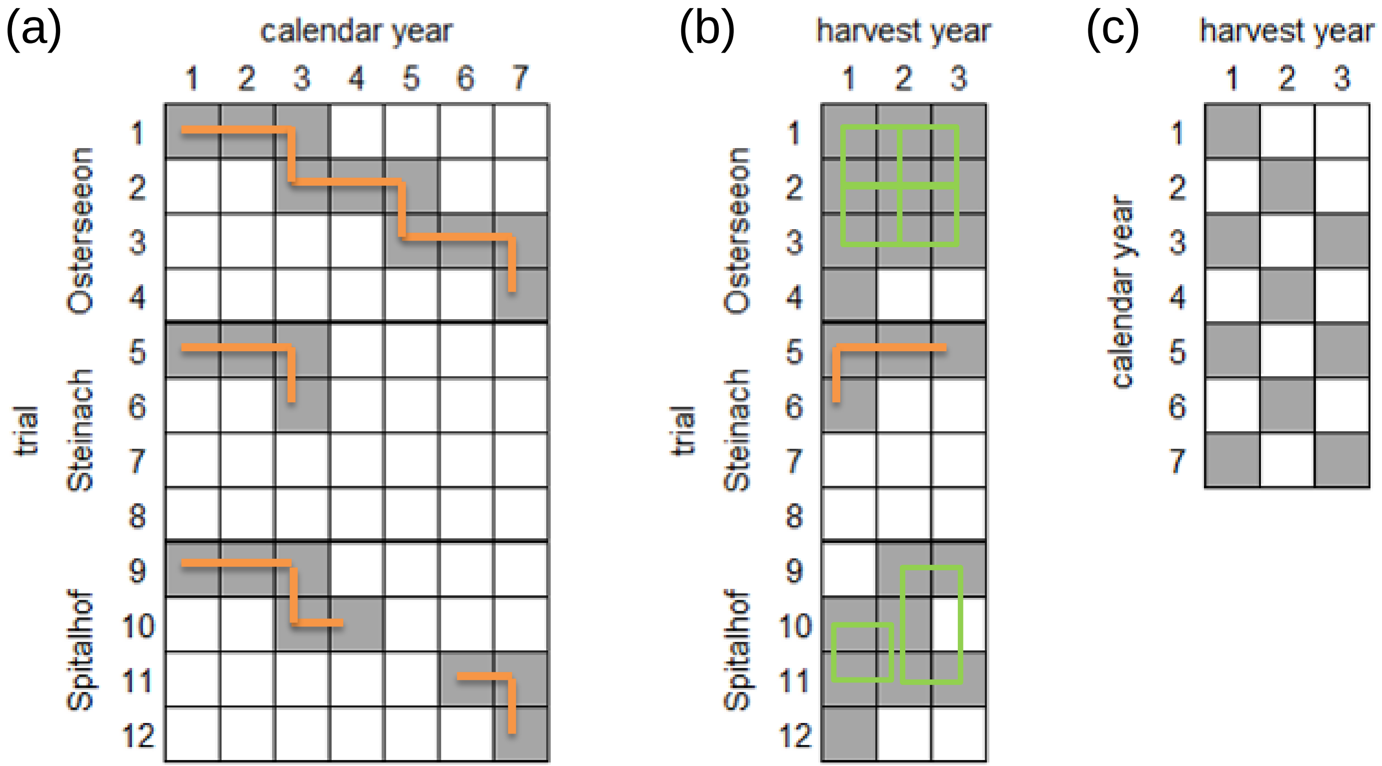
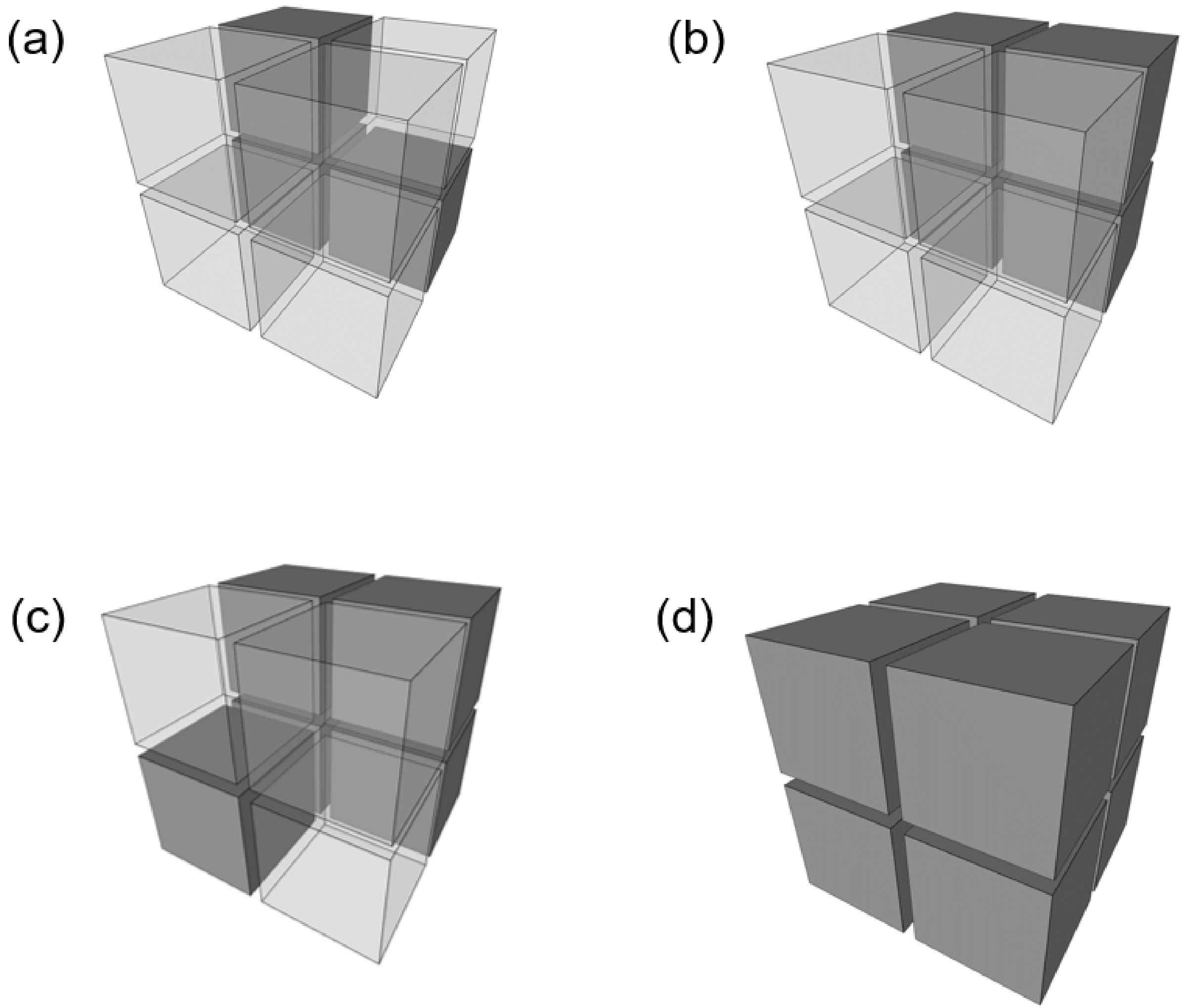


5.1.1. A strategy for year effects in perennial crops

- Octads neccessary for estimating **three-way interactions** (Winer 1962)



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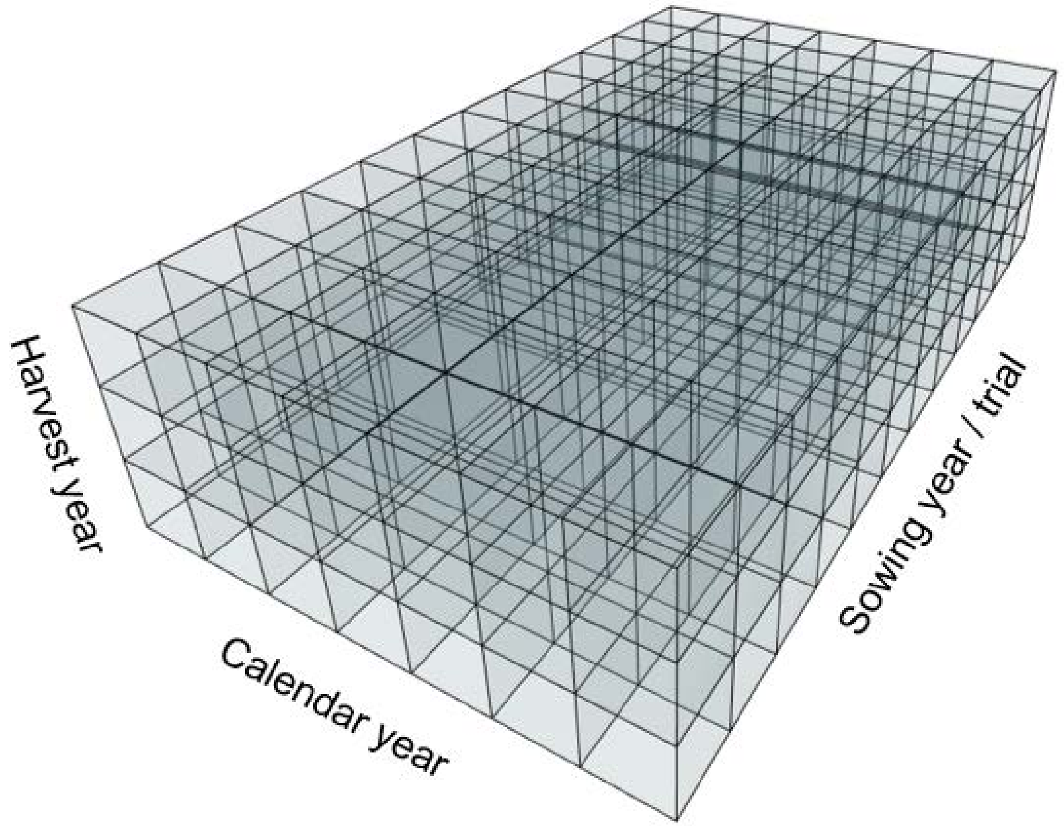
- Octads neccessary for estimating **three-way interactions** (Winer 1962)



5.1.1. A strategy for year effects in perennial crops

Estimability of year effects ?

- ANOVA
- If degrees of freedom
    - = 0: eliminate effect from model
    - ≥ 1: effects stay in model

Fixed or random?

- Analogy between effects of years and blocks
- Decision of recovering or not inter-year information with Kenward and Roger (1997) method
- Interactions modelled dependent on status of year factors

Separate or combined effects?

- Comparison of models with separate year effects to a combined-year effect
- Selection criterion: AIC

5.1. Estimation of variance components

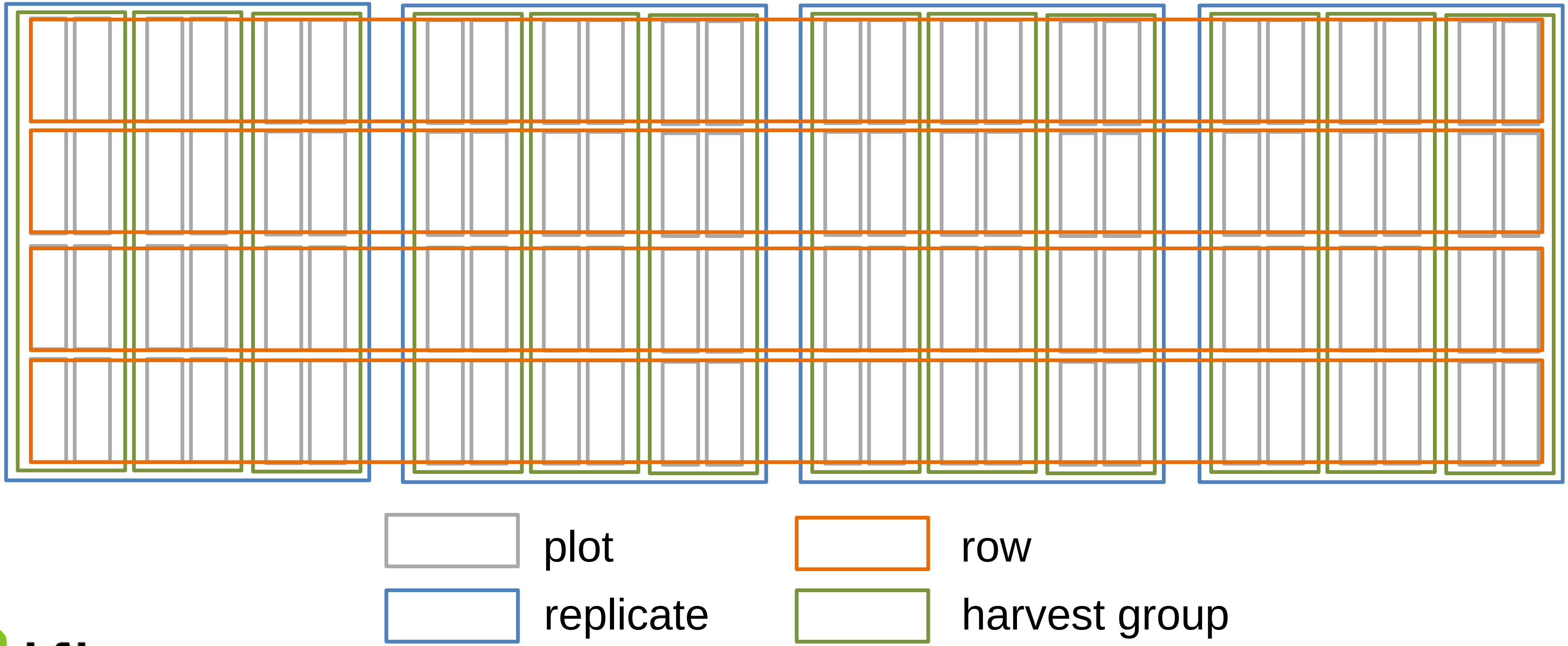
$$\mathrm{M} = \mu + (HG/C) \times \mathrm{HY} \times \mathrm{T} \; + \mathrm{e}$$

where

M	ME in MJ kg^{-1} of a specific cultivar nested in harvest group in a specific trial, cut and harvest year
μ	overall main effect
(HG/C)	fixed effect for cultivar nested within the respective harvest group
HY	fixed effect for harvest year
T	fixed effect for trial (sowing year)
e	random residual error effect of $\mathrm{M}, N(0, \sigma_e^2)$

5.1. Estimation of variance components

Trial design



5.1. Estimation of variance components

$$\mathrm{M} = \mu + (HG/C) \times \mathrm{HY} \times \mathrm{T} : \mathbf{T} \cdot \mathbf{ROW} \cdot \boldsymbol{HY} + \mathbf{T} \cdot \mathbf{REP} \cdot \boldsymbol{HY} + \mathbf{T} \cdot \mathbf{REP} \cdot \mathbf{HG} \cdot \boldsymbol{HY} + \mathrm{e}$$

where

M	ME in MJ kg^{-1} of a specific cultivar nested in harvest group in a specific trial, replicate, cut and harvest year
μ	overall main effect
(HG/C)	fixed effect for cultivar nested within the respective harvest group
HY	fixed effect for harvest year
T	fixed effect for trial (sowing year)
$\mathbf{T} \cdot \mathbf{ROW} \cdot \boldsymbol{HY}$	random effect of the row nested in trial and harvest year, $N(0, \sigma^2_{row})$
$\mathbf{T} \cdot \mathbf{REP} \cdot \boldsymbol{HY}$	random effect of the replicate nested within trial and harvest year, $N\left(0, \sigma^2_{rep}\right)$
$\mathbf{T} \cdot \mathbf{REP} \cdot \mathbf{HG} \cdot \boldsymbol{HY}$	random effect for main plot nested within row and trial and harvest year, $N\left(0, \sigma^2_{hg}\right)$
e	random residual error effect of $\mathrm{M}, N(0, \sigma^2_e)$

5.1. Estimation of variance components

Site	Covariance parameter	Estimate
Osterseeon	$\mathbf{T} \cdot \mathbf{ROW} \cdot \boldsymbol{HY}$	0.1941
Osterseeon	$\mathbf{T} \cdot \mathbf{REP} \cdot \boldsymbol{HY}$	0
Osterseeon	$\mathbf{T} \cdot \mathbf{REP} \cdot \mathbf{HG} \cdot \boldsymbol{HY}$	4.7051
Osterseeon	e	1.8173
Spitalhof	$\mathbf{T} \cdot \mathbf{ROW} \cdot \boldsymbol{HY}$	0
Spitalhof	$\mathbf{T} \cdot \mathbf{REP} \cdot \boldsymbol{HY}$	0.09990
Spitalhof	$\mathbf{T} \cdot \mathbf{REP} \cdot \mathbf{HG} \cdot \boldsymbol{HY}$	0.2785
Spitalhof	e	1.4103
Steinach	$\mathbf{T} \cdot \mathbf{ROW} \cdot \boldsymbol{HY}$	0.2036
Steinach	$\mathbf{T} \cdot \mathbf{REP} \cdot \boldsymbol{HY}$	0.09729
Steinach	$\mathbf{T} \cdot \mathbf{REP} \cdot \mathbf{HG} \cdot \boldsymbol{HY}$	0
Steinach	e	2.8464

5.2. Simulation of alternative designs

1. Estimation of variance components

↓

2. Simulation of alternative designs

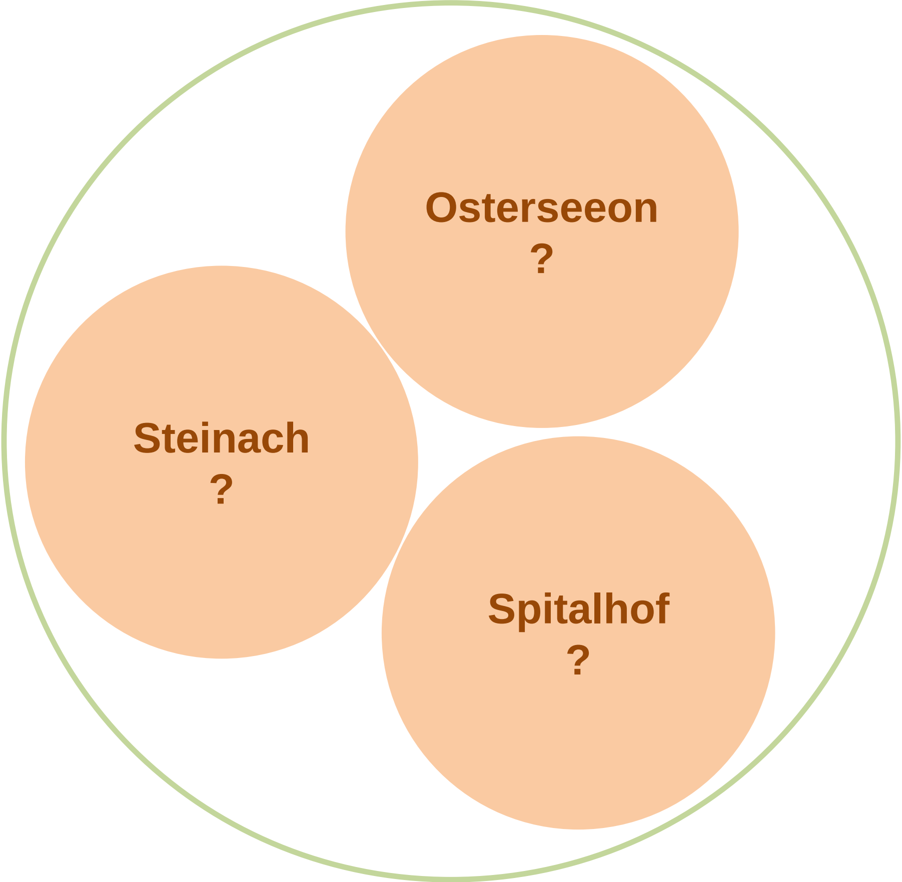
↓

3. Comparison of designs

- With variance components fixed to the values obtained in step 1

5.2. Simulation of alternative designs

Osterseeon	Spitalhof	Steinach	Sum
4	1	1	6
3	1	2	6
3	2	1	6
2	1	3	6
2	2	2	6
2	3	1	6
1	1	4	6
1	2	3	6
1	3	2	6
1	4	1	6
3	1	1	5
2	1	2	5
2	2	1	5
1	1	3	5
1	2	2	5
1	3	1	5
2	1	1	4
1	1	2	4
1	2	1	4
1	1	1	3



≤ 6 samples per cultivar and cut

5.2. Simulation of alternative designs

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2	3	1	6
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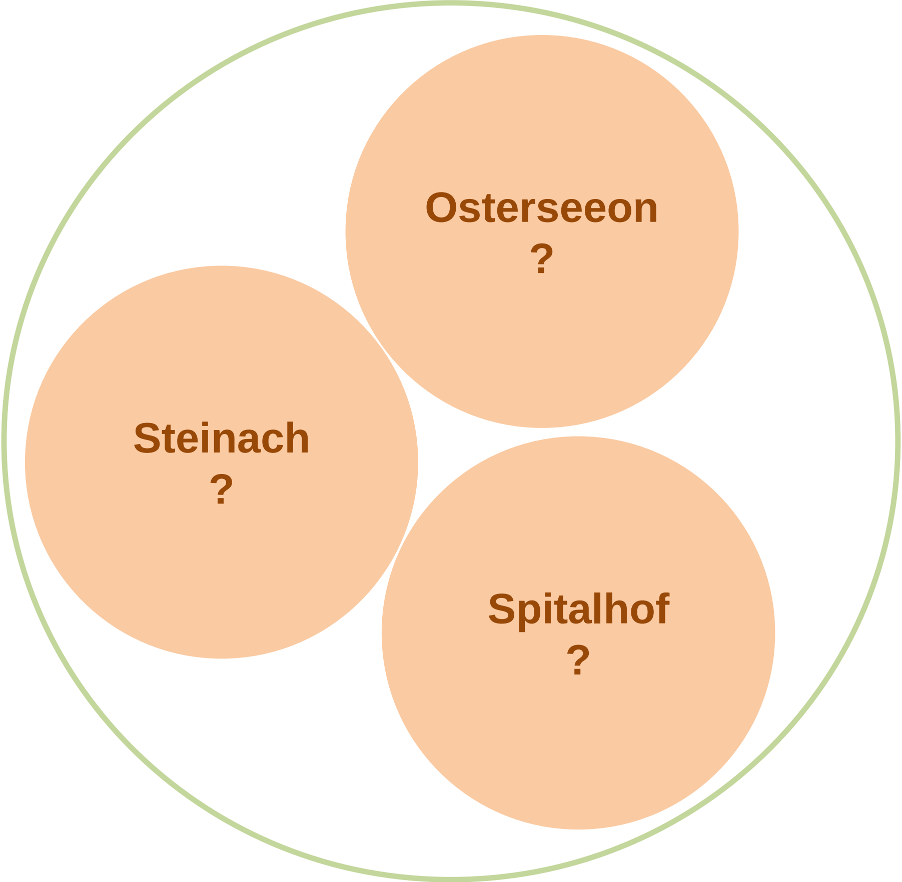
1	2	3	6
1	3	2	6
1	4	1	6
3	1	1	5
2	1	2	5
2	2	1	5
1	1	3	5
1	2	2	5
1	3	1	5
2	1	1	4
1	1	2	4
1	2	1	4
1	1	1	3



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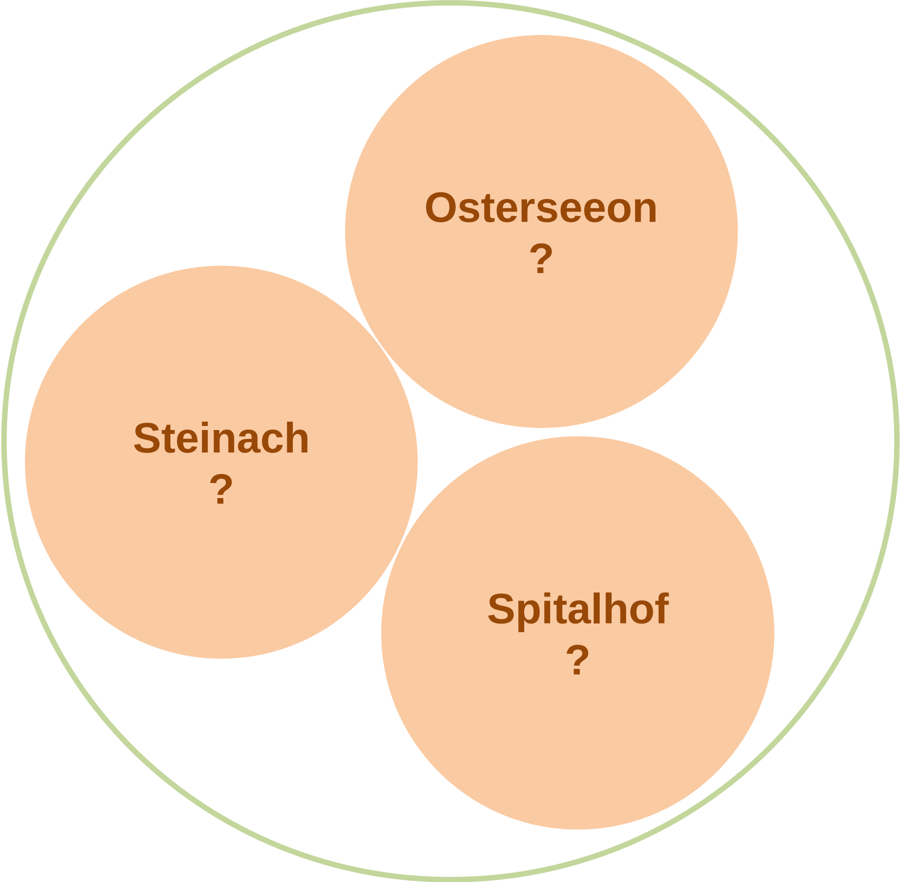

≤ 6 samples per cultivar and cut

5.3. Comparison of designs

1. Estimation of variance components

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2. Simulation of alternative designs

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3. Comparison of designs

- Measurement of statistical power of alternative designs (Stroup 2002)
- Selection of most precise design

6. Concluding remarks

- Focus was on the allocation of quality samples in post-registration trials.
- Perennial crops like perennial ryegrass have unique modelling characteristics, for which the proposed strategy provides guidelines.
- The identified optimal design will enable cultivar evaluations to incorporate quality information without exceeding sample limits.

Fig. 1: Perennial ryegrass in plot trial, S. Hartmann, LfL

Thank you for your attention!

Fig. 1: Perennial ryegrass in plot trial, S. Hartmann, LfL

References

- Bradu, Dan; Gabriel, K. R. (1974): Simultaneous Statistical Inference on Interactions in Two-Way Analysis of Variance. In *Journal of the American Statistical Association* 69 (346), pp. 428–436.
- Graf, Rudolf; Michel, Volker; Roßberg, Dietmar; Neukampf, Ralf (2009): Definition pflanzenartspezifischer Anbaugebiete für ein regionalisiertes Versuchswesen im Pflanzenbau. 247-253 Pages / *Journal für Kulturpflanzen*, Vol. 61 No. 7 (2009). DOI: 10.5073/JFK.2009.07.02.
- Kenward, Michael G.; Roger, James H. (1997): Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. In *Biometrics* 53 (3), p. 983. DOI: 10.2307/2533558.
- Nelder, J. A. (1977): A Reformulation of Linear Models. In *Journal of the Royal Statistical Society. Series A (General)* 140 (1), p. 48. DOI: 10.2307/2344517.
- Piepho, H.-P.; Eckl, T. (2014): Analysis of series of variety trials with perennial crops. In *Grass and Forage Science* 69 (3), pp. 431–440. DOI: 10.1111/gfs.12054.
- Searle, S. R. (1987): Linear models for unbalanced data. New York: Wiley (Wiley series in probability and mathematical statistics. Applied probability and statistics).
- Stroup, W.W. (2002): Power analysis based on spatial effects mixed models: A tool for comparing design and analysis strategies in the presence of spatial variability - Journal of Agricultural, Biological and Environmental Statistics. Springer. Available online at https://link.springer.com/article/10.1198/108571102780, checked on 1/8/2025.
- Winer, B. J. (1962): Statistical principles in experimental design. New York: McGraw-Hill Book Company.