

The comparison of three approaches (ANOVA, ANCOVA and PIP) to the analysis of oilseed rape trials

B. Zawieja¹, C. Flachenecker², W. Pilarczyk¹, T. Mikulski²

¹Department of Mathematical and Statistical Methods,
Poznań University of Life Sciences

²Norddeutsche Planzenzucht Hans-Georg Lemke KG

Preliminary notes

In field trials experimenter often copes (among others) with such problems that can destroy truthfulness of obtained results, as:

- Edge effects
- Interplot competitions (interference)
- Trends in soil productivity

The influence of some of these „difficulties” on results can be (to some extent) constrained by use appropriate experimental designs or mathematical model of observation.

In our presentation we try to show, how different establishing of oil-seed trials and different methods of analysis can change the obtained results.

Data

The results of 10 trials conducted in years 2018-2019 at:

- Two locations in Germany: Leutewitz and Hohenlieth
- Two locations in Poland: Słupia and Głubczyce
- One location in Czech Republic: Humpolec

Seven varieties were tested (three tall – namely T1, T2, T3, three short – called S1, S2, S3 and one of medium height M)

Plot sizes slightly differed from location to location and were:

- 10m² at Humpolec, 13.3m² at Leutewitz, 12.8m² at Hohenlieth and 13m² at Słupia and Głubczyce.

Therefore all calculations were performed after expression all results in dt/ha

Designs of trials

All trials were performed in a special designs in which besides ordinary plots (called single plots), additional wider plots were introduced, split into three subplots (similarly as in split-plot design).

Each tested variety appeared both in single plots and in three subplots of wider plots.

All the measurements were made in all single plots and in all subplots of wider plots.

Central plot of wider plot is called “plot in plot”. Two side subplots are called double plots.

In fact three types of plots are recognized. It is illustrated on the next slide.

Meaning of single, double and PIP

What is single, double and plot in plot (PIP) ?

	Variety a	Variety b			Variety c	Variety a			
.....	single	double	PIP	double	single	double	PIP	double

Example of randomization

types of varieties

A, B, C – short varieties, D – variety of medium height

E, F, G – tall varieties

Location	Glubczyce															
	border	variety	variety	variety	variety	variety	variety	variety	variety	variety	variety	variety	variety	border		
7	B	D	C	C	C	G	E	E	E	A	B	B	B	D	single	
6	B	C	A	A	A	F	G	G	G	D	B	B	B	C	PIP	
5	C	E	D	D	D	F	A	A	A	G	C	C	C	E	double	
4	F	A	A	A	G	C	C	C	E	D	D	D	F	A		
3	B	E	E	E	G	A	A	A	C	F	F	F	B	E		
2	G	B	F	F	F	E	C	C	C	D	G	G	G	B		
1	B	G	G	G	F	D	D	D	A	E	E	E	B	G		
Y/X	1	2	3	4	5	6	7	8	9	10	11	12	13	14		

Assumption, expectations

All our analyses were performed independently for measurements from three types of plots, namely PIP, single and double. **We assumed** (I expect you agree with me) that the results from PIP are closest to the true yielding capacity of tested varieties. **We wanted** to check which other treatment means are closer to PIP means.

We performed following analyses:

- ANOVA from PIP,
- ANOVA from single plots (typical analysis in variety testing for VCU),
- ANOVA for data from double plots,
- ANCOVA for data from single plots with use differences between plant heights as concomitant variable.

The comparison of such ANOVA and ANCOVA means in cereal trials was performed 30 yers ago by Pilarczyk(1992).

In all versions of analysis there were significant differences among treatments.

Calculations

All calculations were performed using the R – free software environment for statistical computing and graphics.

To fit linear models we used **lm** procedure from **stats** package (This procedure can be used for both ANOVA and ANCOVA).

The Least squares means were calculated using the **lsmeans** procedure from **lsmeans** package.

The results from PIP and SINGLE

	ANOVA					PIP					
	2018					2019					
	Glub	Hohe	Hump	Leut	Slup	Glub	Hohe	Hump	Leut	Slup	mean
Medium	55.077	50.208	41.067	47.419	66.179	37.622	54.316	34.000	49.699	32.100	46.77
Short1	63.282	50.000	49.733	49.248	67.538	44.767	55.625	46.033	51.404	29.689	50.73
Short2	62.333	43.854	49.967	42.807	65.821	39.367	51.172	33.700	45.470	31.300	46.58
Short3	55.205	50.260	55.233	45.890	64.667	45.833	60.078	44.400	52.669	35.367	50.96
Tall1	70.256	52.057	49.900	37.870	67.615	38.467	53.477	40.525	41.579	26.800	47.85
Tall2	75.667	53.021	48.233	46.967	65.718	39.933	57.539	42.350	47.494	27.156	50.41
Tall3	64.051	49.792	48.200	40.075	63.974	35.867	53.047	38.300	41.404	25.200	45.99
	ANOVA					SINGLE					
	Glub	Hohe	Hump	Leut	Slup	Glub	Hohe	Hump	Leut	Slup	mean
Medium	61.410	51.797	45.833	48.045	61.282	38.067	54.102	40.400	50.301	34.033	48.53
Short1	58.846	46.641	54.700	52.732	66.205	44.900	58.620	41.133	49.273	32.467	50.55
Short2	53.897	38.984	51.033	38.521	64.897	37.244	45.781	35.967	40.100	22.178	42.86
Short3	55.538	53.490	49.533	42.707	70.333	41.833	54.948	39.050	50.094	33.333	49.09
Tall1	73.538	53.438	46.433	40.025	71.692	42.267	59.961	39.533	43.308	33.767	50.40
Tall2	78.487	55.469	52.867	47.870	62.949	40.450	65.742	52.150	52.381	28.550	53.69
Tall3	68.128	49.063	47.600	40.652	64.154	34.750	45.625	37.750	38.759	28.311	45.48

The difference between PIP and SINGLE

	Glub	Hohe	Hump	Leut	Slup	Glub	Hohe	Hump	Leut	Slup
Medium	-6,333	-1,589	-4,767	-0,627	4,897	-0,444	0,215	-6,400	-0,602	-1,933
Short1	4,436	3,359	-4,967	-3,484	1,333	-0,133	-2,995	4,900	2,130	-2,778
Short2	8,436	4,870	-1,067	4,286	0,923	2,122	5,391	-2,267	5,370	9,122
Short3	-0,333	-3,229	5,700	3,183	-5,667	4,000	5,130	5,350	2,575	2,033
Tall1	-3,282	-1,380	3,467	-2,155	-4,077	-3,800	-6,484	0,992	-1,729	-6,967
Tall2	-2,821	-2,448	-4,633	-0,902	2,769	-0,517	-8,203	-9,800	-4,887	-1,394
Tall3	-4,077	0,729	0,600	-0,576	-0,179	1,117	7,422	0,550	2,644	-3,111
	for short varieties				for tall varieties					
	PIP>SINGLE		20 cases		PIP>SINGLE		9 cases			
	PIP<SINGLE		10 cases		PIP<SINGLE		21 cases			
	max difference		9.122		max difference		-9,8			
Average of absolute differences				3,33						

Difference between PIP and ANCOVA SINGLE

	ANCOVA means for SINGLE																
	2018					2019											
	Glub	Hohe	Hump	Leut	Slup	Glub	Hohe	Hump	Leut	Slup	mean						
Medium	63,592	52,442	45,783	47,817	61,175	38,519	56,074	42,037	50,402	34,338	49,22						
Short1	56,950	46,201	55,063	52,706	65,396	45,420	60,483	43,043	52,445	33,574	51,13						
Short2	49,248	39,040	51,387	40,462	64,857	37,935	49,847	40,705	44,786	23,140	44,14						
Short3	54,780	53,266	49,586	43,796	70,403	41,800	55,633	37,560	50,803	33,295	49,09						
Tall1	73,758	53,641	46,153	38,943	72,047	42,227	56,723	37,493	39,546	32,472	49,30						
Tall2	81,578	54,657	52,332	47,249	63,162	39,858	61,885	47,621	48,831	28,190	52,54						
Tall3	69,940	49,634	47,697	39,579	64,474	34,272	47,923	38,114	38,303	27,433	45,74						
differences between PIP and ANCOVA means for single																	
	Glub	Hohe	Hump	Leut	Slup	Glub	Hohe	Hump	Leut	Slup							
Medium	-8,515	-2,233	-4,717	-0,398	5,005	-0,896	-1,757	-8,037	-0,703	-2,238							
Short1	6,332	3,799	-5,329	-3,458	2,142	-0,653	-4,858	2,991	-1,041	-3,885							
Short2	13,085	4,814	-1,420	2,345	0,964	1,431	1,325	-7,005	0,684	8,160							
Short3	0,425	-3,006	5,647	2,094	-5,736	4,034	4,445	6,840	1,867	2,072							
Tall1	-3,501	-1,583	3,747	-1,074	-4,431	-3,760	-3,246	3,032	2,033	-5,672							
Tall2	-5,911	-1,636	-4,098	-0,281	2,556	0,076	-4,346	-5,271	-1,338	-1,034							
Tall3	-5,888	0,158	0,503	0,496	-0,499	1,595	5,124	0,186	3,101	-2,233							
for short varieties					for tall varieties												
PIP>SINGLE			20 cases		PIP>SINGLE			12 cases									
PIP<SINGLE			10 cases		PIP<SINGLE			18 cases									
max difference			13,085		max difference			-5,911									
Average of absolute differences					3,21												

Comparison of PIP and DOUBLE

	ANOVA					DOUBLE					
	2018					2019					
	Glubczyce	ohenlieth	lumpolec	eutewitz	Slupia	glubczyce	ohenlieth	lumpolec	eutewitz	Slupia	mean
Medium	55,410	48,958	41,350	48,459	66,590	37,533	56,504	38,300	48,816	31,708	47,36
Short1	63,577	49,622	51,283	49,524	67,167	44,292	58,268	42,217	52,368	28,944	50,73
Short2	62,295	44,245	49,383	39,950	64,705	39,033	49,570	35,300	42,904	29,833	45,72
Short3	53,282	50,065	53,200	44,749	62,603	45,483	60,091	42,183	50,489	36,600	49,87
Tall1	75,538	53,216	50,667	38,872	72,321	40,478	59,141	39,675	42,857	27,992	50,08
Tall2	76,551	52,500	48,400	49,223	66,077	39,083	63,223	45,750	48,734	29,811	51,94
Tall3	65,115	50,859	50,650	40,201	63,782	34,467	55,703	37,113	41,078	29,044	46,80
differences between PIP and ANOVA DOUBLE means											
	2018					2019					
	Glub	Hohe	Hump	Leut	Slup	Glub	Hohe	Hump	Leut	Slup	
Medium	-0,333	1,250	-0,283	-1,040	-0,410	0,089	-2,187	-4,300	0,883	0,392	
Short1	-0,295	0,378	-1,550	-0,276	0,372	0,475	-2,643	3,817	-0,965	0,744	
Short2	0,038	-0,391	0,583	2,857	1,115	0,333	1,602	-1,600	2,566	1,467	
Short3	1,923	0,195	2,033	1,140	2,064	0,350	-0,013	2,217	2,180	-1,233	
Tall1	-5,282	-1,159	-0,767	-1,003	-4,705	-2,011	-5,664	0,850	-1,278	-1,192	
Tall2	-0,885	0,521	-0,167	-2,256	-0,359	0,850	-5,684	-3,400	-1,241	-2,656	
Tall3	-1,064	-1,068	-2,450	-0,125	0,192	1,400	-2,656	1,188	0,326	-3,844	
Average of absolute differences					1,500						

Comparison of means from ANOVA and ANCOVA for single

	2018					2019					
	Glub	Hohe	Hump	Leut	Slup	Glub	Hohe	Hump	Leut	Slup	
Medium	-2,182	-0,645	0,05	0,228	0,107	-0,452	-1,972	-1,637	-0,101	-0,305	
Short1	1,896	0,439	-0,363	0,026	0,809	-0,52	-1,864	-1,909	-3,172	-1,107	
Short2	4,649	-0,056	-0,353	-1,941	0,04	-0,691	-4,066	-4,738	-4,686	-0,962	
Short3	0,758	0,224	-0,053	-1,089	-0,069	0,034	-0,685	1,49	-0,709	0,038	
Tall1	-0,219	-0,203	0,281	1,082	-0,355	0,04	3,238	2,041	3,762	1,295	
Tall2	-3,091	0,812	0,535	0,621	-0,213	0,592	3,857	4,529	3,55	0,36	
Tall3	-1,812	-0,571	-0,097	1,073	-0,32	0,478	-2,298	-0,364	0,457	0,878	
	mean absolute difference			1,23							
	short varieties					tall varieties					
	max difference		-4,738			4,529					

CONCLUSIONS

- 1) Our results confirm that existence of neighbouring influences is a real and serious problem in oil-seed rape trials – see 9.1 dt/ha as maximum difference between PIP mean and SINGLE plot mean;

- 2) The analysis of covariance only partly solves the problem:
 - see increase of ANCOVA means (compared to ANOVA) for short varieties in 20 (out of 30) cases and maximum increase of 4.7dt/ha,
 - see also decrease of ANCOVA means for tall varieties in 18 (out of 30) cases and maximum decrease of 4.5 dt/ha;
 - additional argument towards application of ANCOVA is that average absolute difference between ANCOVA and ANOVA mean values was 1.2 dtha;

Conclusions cont.

3) DOUBLE plot means closer to PIP means (average absolute difference 1.5 dt/ha) than SINGLE plot means (average absolute difference 3.3 dt/ha) suggest application of wider plots;

4) Another possible solution - grouping varieties in groups of similar height and application of special experimental design similar to split-plot design (larger plots for groups, small plots for varieties).

Please note that in such design there is hierarchical classification (varieties within groups) while in split-plot design the cross classification appears. Details of analysis of such data are in a paper (**unfortunately in Polish**) by Ukalska J. and Pilarczyk W. (2002), Colloquium Biometricum 32, pp.51-66.

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