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LINEAR STATISTICAL INFERENCE ON THE CHLOROPHYLL CONTENT IN DIFFERENT STAGES OF THE MAIZE VARIETY ‘ANJOU 258’

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Summary

The paper deals with an additional particular analysis of the four-year study on the chlorophyll content of the maize variety ‘Anjou 258’. The field trial was conducted every year in a split-plot design at the Agricultural Experimental Station in Swadzim (Poland) during 2005-2008. This inference is based on three factor analysis of variance (ANOVA) technique supported by the theory of contrasts. Particular attention is paid to estimation and testing some comparisons among treatment combination effects connected with six doses of urea $CO(NH_2)_2$ and three doses of elemental sulphur through examined series of years.

Kewords and phrases: The maize variety ‘Anjou 258’, nitrogen, elemental sulphur, ANOVA, split-split-plot design, *F* test, contrasts, Bonferroni method, Dunnett test

Classification AMS 2010: 62K10, 62K15

1. Introduction

The aim of the paper is to give some tools for linear statistical inference from field trials conducted in a split-plot design at the Department of Agronomy, Poznań University of Life Sciences, on the fields of the Department of Teaching and Experimental Station in Swadzim during 2005–2008. This inference is based on three factor analysis of variance (ANOVA) technique for the experiment carried out in split-split-plot design. Additionally, the general analysis is supported by an inference based on the theory of (basic) contrasts (c.f. Pearce et al., 1974).

Details connected with methodology of experiments carried out in a split-split-plot design may be found in many textbooks and monographs (e.g. Gomez and Gomez, 1984; Hinkelmann and Kempthorne, 1994, Thomas, 2006). See also Ambroży-Deręgowska et al. (2015).

In this paper we adapt the analysis of variance (ANOVA) for data from split-split-plot experiment (cf. Ambroży and Mejza, 2011). We have performed a three factor analysis of split-split-plot design in which effects of the years and other factors are treated as fixed. Therefore, further inference concerning environments (climate and soil conditions) is limited to environments similar to the years of the experiments. Also, basic contrasts (c.f. Pearce et al., 1974) and any contrasts were used in the analysis. Particular attention is paid to estimation and testing some comparisons among treatment combination effects connected with six doses of urea through examined series of years and three doses of sulphur. Other analyses of this experiment can be find in Szulc et al. (2012).

2. Materials and methods

Field trial. Every year the field experiment was conducted in a split-plot design with two tested factors, whole plots – nitrogen, subplots – sulphur in four field replications (r = 4). In the experiment effects of six doses of urea $CO(NH_2)_2$ (with nitrogen: N1 – 0, N2 – 30, N3 – 60, N4 – 90, N5 – 120 and N6 – 150 kg N ha^{-1}) and three doses of elemental sulphur (S1 – 0, S2 – 20, S3 – 40 kg S ha^{-1}), inter alia, on the chlorophyll content in different stages of the maize variety ‘Anjou 258’ (FAO 260–270) were investigated. The field experiment was conducted on the podsolic soil, light clay sand grade, shallowly deposited on the light clay belonging to a good rye complex. According to the international classification WRB (World reference base for soil resources 2014), the examined soils should be classified as Phaeozems (Haplic Phaeozems), while according to the US Soil Taxonomy (Soil taxonomy 1999) as Mollisols (Typic Endoaquolls). The abundance of basic macronutrients (P, K, Mg) in the soil in each year of the study influenced at the average level, while its acidity ranged from 5.8 in 2008 to 6.1 in 2006. Total sulphur ranged from 0.16 in 2006 to 0.19 g kg^{-1} soil in 2008. Estimation of Mg in the soil was performed by the Schachtschabel method, while K and P were determined by the Egner-Riehm method. Maize was sown in row spacing of 70 cm at a depth of 4–5 cm. The content of humus in the arable layer (0–25 cm) in the years of research ranged from 1.41% to 1.46%. Phosphorus at a rate of 80 kg P_2O_5 ha^{-1} was used in the form of the granulated triple superphosphate and potassium was used at a rate of 120 kg K_2O ha^{-1} in the form of potassium salt.

Meteorological conditions are described in Szulc et al. (2012).

Chlorophyll. The content of chlorophyll *a*, *b*, *a*+*b* was determined at the 5–6 leaf stage (BBCH 15/16) and during the ear blooming stage (BBCH 67). For a detailed description of the method for chlorophyll determination in maize leaf blades, please refer to the earlier paper (Szulc 2009).

Chlorophyll content was determined using a spectrophotometer (Spekol type) at the appropriate wavelength. For chlorophyll *a*, the measurement of absorbance of the extract was performed at the wavelength of 663 nm and for chlorophyll *b* at the wavelength of 645 nm. The content of chlorophyll *a*, chlorophyll *b* and total chlorophyll *a*+*b* was calculated using the formulas from the paper by Arnon (1949). The amount of particular pigments was given in $\mu g \cdot g^{-1}$ of the fresh weight: chlorophyll $a = (12.7 \cdot A_{663} - 2.7 \cdot A_{645}) \cdot V \cdot (1000\ W)^{-1}$; chlorophyll $b = (22.9 \cdot A_{645} - 4.7 \cdot A_{663}) \cdot V \cdot (1000\ W)^{-1}$; total $a+b = (20.2 \cdot A_{645} - 8.02 \cdot A_{663}) \cdot V \cdot (1000\ W)^{-1}$, where A_w is the absorbance at a given wavelength *w*, V – the total volume of the extract (cm^3) and W – the weight of a sample (g).

Table 1. Weather conditions during the studied development stages and number of days from sowing to the 5–6 leaf stage and from sowing to ear blooming in the Experimental Unit Swadzim

Specification	Years			
	2005	2006	2007	2008
Sowing date	16 IV	21 IV	20 IV	26 IV
5–6 leaf stage	29 V	26 V	23 V	25 V
Number of days (sowing–5–6 leaves)	43	35	33	29
Sum of rainfall mm (sowing–5–6 leaves)	81.7	65.2	74.8	14.1
Mean air temperature °C (sowing–5–6 leaves)	12.2	13.7	13.9	14.8
Ear blooming stage	23 07	14 07	10 07	14 07
Number of days (sowing–ear blooming)	98	84	81	79
Sum of rainfall (sowing–ear blooming)	179.4	139.2	185.5	88.6
Mean air temperature °C (sowing–ear blooming)	14.9	17.6	16.5	17.7
Number of days (5–6 leaves–ear blooming)	55	49	48	50
Sum of rainfall (5–6 leaves–ear blooming)	97.3	74.0	110.7	74.5
Mean air temperature (5–6 leaves–ear blooming)	13.5	15.6	15.2	16.2

3. Results

ANOVA

Analysis of variance performed for six traits separately gave results described partly in Table 2. They indicate a statistically significant influence of the year effects on the mean chlorophyll content in different stages of maize variety ‘Anjou 258’.

Table 2. Mean squares of split-split-plot ANOVA for the chlorophyll content in different stages of maize variety ‘Anjou 258’

Source of variation	d.f.	Chlorophyll					
		a stage_56	*a* stage_bl	*b* stage_56	*b* stage_bl	*a*+*b* stage_56	*a*+*b* stage_bl
Blocks	3	0.027	0.036	0.005	0.002	0.023	0.052
Years (Y)	3	8.883**	10.452**	0.169**	0.183**	11.244**	13.376**
Error 1	9	0.083	0.281	0.005	0.008	0.102	0.383
Nitrogen (N)	5	0.549**	1.198**	0.008**	0.018**	0.792**	1.512**
Y×N	15	0.026	0.254**	0.001	0.007**	0.035	0.337**
Error 2	60	0.044	0.073	0.001	0.002	0.056	0.097
Sulphur (S)	2	0.019	0.140	0.002	0.006*	0.028	0.207
Y×S	6	0.052	0.064	0.002	0.003	0.101*	0.092
N×S	10	0.058*	0.127*	0.002	0.003*	0.060	0.166*
Y×N×S	30	0.047*	0.127**	0.001	0.003**	0.063*	0.166**
Error 3	144	0.029	0.061	0.001	0.002	0.036	0.081

stage_56 – 5–6 leaf stage (BBCH 15/16); stage_bl – ear blooming stage (BBCH 67)

* $p < 0.05$; ** $p < 0.01$

During the years considered the results indicate a statistically significant influence of nitrogen effects on the mean chlorophyll content only for ear blooming stages (*a*, *b*, *a*+*b*) of this maize. It was also found a significant interaction between nitrogen and the years for these traits and three-way interaction of nitrogen with the years and sulphur (apart of one trait – chloropyll *b* stage 56).

The results presented in Table 2 indicate nonsignificant influence of sulphur effect on the mean chlorophyll content (apart of one trait – chloropyll *b* stage_bl) and nonsignificant two-way interaction between years and sulphur (apart of one trait – chloropyll *a*+*b* stage_bl). These are statistically significance at the level $p < 0.05$.

Basic contrasts

In the paper basic contrasts define comparisons among main effects of the years considered, the doses of nitrogen effects and the doses of sulphur effects, and also interaction effects between them. They provide supporting techniques for ANOVA. Procedures of creating a set of basic contrasts and an application of them in a statistical analysis can be found in many papers (e.g. Ambroży and Mejza, 2012, Ambroży-Deręgowska et al., 2015). One of the method of creating the set of basic contrasts uses eigenvectors of information matrices for a split-split-plot design. In this paper we propose a structure of vectors defining contrasts as follows.

The orthonormal (with respect to replications, $r = 4$) eigenvectors can have (for example) the form:

$$\mathbf{p}_h = \frac{1}{\sqrt{4}}\mathbf{a}_j \otimes \mathbf{b}_k \otimes \mathbf{c}_l ,$$

for $h = 18(j-1) + 3(k-1) + l$; $j = 1, 2, 3, 4$; $k = 1, 2, \ldots 6$; $l = 1, 2, 3$,

where

$$\begin{array}{lll}
\mathbf{a}_1 = [1, -1, 0, 0]'/\sqrt{2} & \mathbf{b}_1 = [1, -1, 0, 0, 0, 0]'/\sqrt{2} & \mathbf{c}_1 = [1, -1, 0]'/\sqrt{2} \\
\mathbf{a}_2 = [1, 1, -2, 0]'/\sqrt{6} & \mathbf{b}_2 = [1, 1, -2, 0, 0, 0]'/\sqrt{6} & \mathbf{c}_2 = [1, 1, -2]'/\sqrt{6} \\
\mathbf{a}_3 = [1, 1, 1, -3]'/\sqrt{12} & \mathbf{b}_3 = [1, 1, 1, -3, 0, 0]'/\sqrt{12} & \mathbf{c}_3 = [1, 1, 1]'/\sqrt{3} \\
\mathbf{a}_4 = [1, 1, 1, 1]'/2 & \mathbf{b}_4 = [1, 1, 1, 1, -4, 0]'/\sqrt{20} & \\
 & \mathbf{b}_5 = [1, 1, 1, 1, 1, -5]'/\sqrt{30} & \\
 & \mathbf{b}_6 = [1, 1, 1, 1, 1, 1]'/\sqrt{6} &
\end{array}$$

are orthonormal vectors connected with the years (**a**), the doses of nitrogen (**b**) and the doses of sulphur (**c**).

These eigenvectors, under certain conditions (e.g. Ambroży-Deręgowska et al., 2015), define the basic contrasts, which coefficients are calculated by $4\mathbf{p}_h$, $h = 1, 2, \ldots, 71$. In Table 3 we present stratum estimates of them.

Table 3. Stratum estimates of the basic contrasts for the traits considered

Indexes				Type of contrasts	No of stratum #	Chlorophyll					
						a stage_56	*a* stage_bl	*b* stage_56	*b* stage_bl	*a+b* stage_56	*a+b* stage_bl
h	*j*	*k*	*l*								
1	1	1	1	Y×N×S	(3)	0.0773	0.3759	0.0466	0.0607	0.1066	0.4372
2	1	1	2	Y×N×S	(3)	0.3261	-0.0331	0.0049	0.0162	0.3276	-0.0170
3	1	1	3	Y × N	(2)	0.0714	0.0387	-0.0214	0.0371	0.0736	0.0759
4	1	2	1	Y×N×S	(3)	0.2922	-0.4497	-0.0282	-0.0324	0.2955	-0.4828
5	1	2	2	Y×N×S	(3)	-0.1669	-0.0009	-0.0028	-0.0134	-0.1823	-0.0143
6	1	2	3	Y×N	(2)	0.1852	-0.2847	0.0119	-0.0311	0.2288	-0.3163
7	1	3	1	Y×N×S	(3)	-0.3462	0.1263	-0.0341	0.0158	-0.3967	0.1423
8	1	3	2	Y×N×S	(3)	-0.1618	0.0003	0.0080	-0.0076	-0.1589	-0.0073
9	1	3	3	Y×N	(2)	0.1334	-0.1623	-0.0205	0.0200	0.1663	-0.1426
10	1	4	1	Y×N×S	(3)	-0.2631	0.5832	-0.0606	0.0901	-0.3141	0.6743
11	1	4	2	Y×N×S	(3)	-0.0517	0.8709	-0.0061	0.1305	-0.0575	1.0028
12	1	4	3	Y×N	(2)	0.1676	-0.4463	0.0189	-0.0616	0.2120	-0.5087
13	1	5	1	Y×N×S	(3)	0.4235	-0.1973	0.0231	-0.0664	0.4703	-0.2640
14	1	5	2	Y×N×S	(3)	0.2042	0.3289	0.0101	0.0617	0.2248	0.3911
15	1	5	3	Y×N	(2)	-0.0131	-0.1180	-0.0025	-0.0219	-0.0182	-0.1402
16	1	6	1	Y×S	(3)	-0.0997	-0.1305	-0.0202	-0.0454	-0.1403	-0.1761
17	1	6	2	Y×S	(3)	-0.1083	-0.0014	-0.0026	-0.0140	-0.1137	-0.0153
18	1	6	3	Y	(1)	0.8979	1.2313	0.0786	0.2209	1.0566	1.4543
19	2	1	1	Y×N×S	(3)	0.3567	-0.2286	0.0586	-0.0508	0.6215	-0.2798

Table 3. continued

20	2	1	2	Y×N×S	(3)	-0.1007	-0.0018	-0.0286	-0.0105	-0.0143	-0.0122
21	2	1	3	Y×N	(2)	0.0783	-0.0405	0.0566	0.0421	0.2813	0.0015
22	2	2	1	Y×N×S	(3)	-0.1423	0.0922	-0.0357	-0.0165	-0.0832	0.0759
23	2	2	2	Y×N×S	(3)	-0.2884	-0.2704	-0.0131	0.0100	-0.2296	-0.2608
24	2	2	3	Y×N	(2)	0.0437	-0.3579	0.0180	-0.0542	0.1358	-0.4127
25	2	3	1	Y×N×S	(3)	0.0452	-0.2576	-0.0502	0.0048	0.0841	-0.2532
26	2	3	2	Y×N×S	(3)	-0.0666	0.3912	-0.0353	0.0790	-0.0532	0.4709
27	2	3	3	Y×N	(2)	-0.2687	0.0231	0.0429	0.0094	-0.1917	0.0325
28	2	4	1	Y×N×S	(3)	-0.3198	0.3056	0.0025	0.0181	-0.2617	0.3242
29	2	4	2	Y×N×S	(3)	0.0480	-1.0376	0.0152	-0.1805	0.0988	-1.2198
30	2	4	3	Y×N	(2)	0.1713	0.0577	0.0982	0.0367	0.3058	0.0945
31	2	5	1	Y×N×S	(3)	-0.0547	0.5316	0.0297	0.0788	0.0121	0.6113
32	2	5	2	Y×N×S	(3)	0.1455	-0.1138	0.0954	-0.0325	0.2644	-0.1466
33	2	5	3	Y×N	(2)	-0.0712	-1.6652	0.0088	-0.2771	-0.0199	-1.9450
34	2	6	1	Y×S	(3)	0.3185	0.1464	0.0624	0.0163	0.5061	0.1630
35	2	6	2	Y×S	(3)	0.0534	-0.5729	0.0149	-0.1052	0.1351	-0.6791
36	2	6	3	Y	(1)	-4.6747	-4.1267	-0.7063	-0.4288	-5.3421	-4.5623
37	3	1	1	Y×N×S	(3)	0.0842	-0.3143	0.0112	-0.0303	0.0335	-0.3451
38	3	1	2	Y×N×S	(3)	-0.0823	0.2475	-0.0154	0.0206	-0.1362	0.2686
39	3	1	3	Y×N	(2)	0.2259	-0.0758	0.0233	-0.0114	0.1835	-0.0874
40	3	2	1	Y×N×S	(3)	-0.0372	-0.1703	0.0596	0.0165	-0.0303	-0.1541
41	3	2	2	Y×N×S	(3)	-0.1421	-0.1127	0.0027	-0.0118	-0.1576	-0.1247
42	3	2	3	Y×N	(2)	-0.0006	0.5682	-0.0004	0.0385	-0.0464	0.6077

Table 3. continued

43	3	3	1	Y×N×S	(3)	0.2358	0.1722	0.0433	0.0218	0.2578	0.1942
44	3	3	2	Y×N×S	(3)	0.0725	-0.2540	0.0128	-0.0420	0.0711	-0.2964
45	3	3	3	Y×N	(2)	0.2992	0.0472	0.0115	0.0365	0.2664	0.0838
46	3	4	1	Y×N×S	(3)	0.1916	0.2003	0.0421	-0.0373	0.2081	0.1633
47	3	4	2	Y×N×S	(3)	0.3559	-0.1177	0.0522	-0.0139	0.3958	-0.1318
48	3	4	3	Y×N	(2)	0.1054	0.4892	0.0296	0.0358	0.1071	0.5259
49	3	5	1	Y×N×S	(3)	-0.0131	-0.1358	-0.0105	-0.0280	-0.0509	-0.1640
50	3	5	2	Y×N×S	(3)	-0.3385	0.0914	-0.0563	0.0155	-0.4098	0.1070
51	3	5	3	Y×N	(2)	-0.1737	0.0841	-0.0381	0.0560	-0.2256	0.1402
52	3	6	1	Y×S	(3)	-0.4280	-0.1319	-0.0837	-0.0420	-0.5441	-0.1742
53	3	6	2	Y×S	(3)	-0.0185	0.0466	0.0108	0.0045	-0.0297	0.0512
54	3	6	3	Y	(1)	-1.9973	-3.5793	0.0461	-0.5620	-2.0191	-4.1473
55	4	1	1	N×S	(3)	0.0093	0.1723	0.0435	0.0259	-0.0547	0.1985
56	4	1	2	N×S	(3)	0.5720	-0.2211	0.0534	-0.0148	0.5600	-0.2362
57	4	1	3	N	(2)	-0.3472	-0.2016	-0.0726	-0.0522	-0.5348	-0.2542
58	4	2	1	N×S	(3)	0.3684	0.1951	0.0660	0.0730	0.3439	0.2685
59	4	2	2	N×S	(3)	0.2809	0.2192	0.0423	0.0077	0.2926	0.2272
60	4	2	3	N	(2)	-0.4582	-1.0109	-0.0406	-0.1456	-0.5783	-1.1581
61	4	3	1	N×S	(3)	0.0429	0.5927	-0.0470	0.0698	-0.0417	0.6635
62	4	3	2	N×S	(3)	-0.1176	-0.3345	0.0131	-0.0705	-0.1296	-0.4055
63	4	3	3	N	(2)	-1.2060	-1.3516	-0.1359	-0.1413	-1.4214	-1.4952
64	4	4	1	N×S	(3)	-0.0655	-0.1530	-0.0488	-0.0477	-0.1595	-0.2010
65	4	4	2	N×S	(3)	-0.1069	-0.6378	-0.0280	-0.0896	-0.1573	-0.7284

Table 3. continued

66	4	4	3	N	(2)	-0.9634	-1.6852	-0.1131	-0.1923	-1.1269	-1.8803
67	4	5	1	N×S	(3)	-0.0121	0.4377	0.0252	0.0532	-0.0341	0.4916
68	4	5	2	N×S	(3)	-0.0436	-0.1528	-0.0169	-0.0035	-0.0856	-0.1566
69	4	5	3	N	(2)	-0.1771	0.5104	-0.0225	0.1067	-0.2234	0.6180
70	4	6	1	S	(3)	-0.0455	-0.3353	-0.0519	-0.0787	-0.1523	-0.4146
71	4	6	2	S	(3)	0.1910	0.4103	0.0263	0.0806	0.1795	0.4916

(1) – the whole plot stratum, (2) – the subplot stratum, (3) – the sub-subplot stratum.

Table 4. Detailed analysis based on the basic contrasts of type N (nitrogen) (for chlorophyll *b* stage_bl)

Stratum (2) - Analysis of subplots					
Source of variation	df	SS	MS	*F*	*p*
Contrasts of type N including:	5	0.0922	0.0184	8.6635^{**}	0.0000
$\mathbf{c}'_{57}\boldsymbol{\tau}$	1	0.0027	0.0027	1.2811	0.2622
$\mathbf{c}'_{60}\boldsymbol{\tau}$	1	0.0212	0.0212	9.9507^{**}	0.0025
$\mathbf{c}'_{63}\boldsymbol{\tau}$	1	0.0200	0.0200	9.3794^{**}	0.0033
$\mathbf{c}'_{66}\boldsymbol{\tau}$	1	0.0370	0.0370	17.3606^{**}	0.0001
$\mathbf{c}'_{69}\boldsymbol{\tau}$	1	0.0114	0.0114	5.3458^{*}	0.0242
Rest	15	0.0973	0.0065		
Error 2	60	0.1278	0.0021		
Total (2) - ***subplots***	80	0.3173			

* $p < 0.05$; ** $p < 0.01$

Table 4 presents the detailed analysis in the subplot stratum (2), in which five contrasts among main effects of nitrogen and 15 interaction contrasts between years and nitrogen (Rest) are estimated with full efficiency. In this work we focused only on those first mentioned contrasts with subscripts 57, 60, 63, 66, 69. Using the estimates from Table 3, we have tested them. It can be seen from Table 4, that we fail to reject the particular hypothesis connected with the contrast $\mathbf{c}'_{57}\boldsymbol{\tau}$ declaring that there is no significant difference between the true average of the chlorophyll content in maize variety ‘Anjou 258’ for the chlorophyll *b* stage_bl, using doses N_1 - 0 and N_2 - 30 kg N ha^{-1}. Other contrasts

($\mathbf{c}'_{60}\boldsymbol{\tau}$, $\mathbf{c}'_{63}\boldsymbol{\tau}$, $\mathbf{c}'_{66}\boldsymbol{\tau}$, $\mathbf{c}'_{69}\boldsymbol{\tau}$) are significant. Probably the particular hypotheses for those contrasts are responsible for the rejection of the general hypothesis connected with main effects of Nitrogen in ANOVA (Table 2). A similar detailed analysis was done for other traits and the results are presented in Table 5.

Any contrasts

Sometimes the basic treatment contrasts are difficult to interpret. Especially in the nonorthogonal designs. In this case, when information matrices have all different eigenvalues, there exists only one unique set of eigenvectors, corresponding to that eigenvalues. More freedom in choosing orthogonal set of eigenvectors of information matrices we have in orthogonal (or balanced) case of a design.

Table 5. Mean squares from detailed analysis for basic contrasts of type N (nitrogen) for chlorophyll content in different stages of maize variety 'Anjou 258'

Source of variation	df	Chlorophyll					
		a stage_56	*a* stage_bl	*b* stage_56	*b* stage_bl	*a*+*b* stage_56	*a*+*b* stage_bl
Contrasts of type N including	5	0.549	1.198	0.008	0.018	0.792	1.512
$\mathbf{c}'_{57}\boldsymbol{\tau}$	1	0.121	0.041	0.005	0.003	0.286*	0.065
$\mathbf{c}'_{60}\boldsymbol{\tau}$	1	0.210*	1.022**	0.002	0.021**	0.334*	1.341**
$\mathbf{c}'_{63}\boldsymbol{\tau}$	1	1.454**	1.827**	0.019**	0.020**	2.020**	2.236**
$\mathbf{c}'_{66}\boldsymbol{\tau}$	1	0.928**	2.840**	0.013**	0.037**	1.270**	3.536**
$\mathbf{c}'_{69}\boldsymbol{\tau}$	1	0.031	0.261	0.001	0.011*	0.050	0.382
Rest	15	0.026	0.254	0.001	0.007	0.035	0.337
Error 2	60	0.044	0.073	0.001	0.002	0.056	0.097

* $p < 0.05$; ** $p < 0.01$

Then it is no problem to find orthogonal set of vectors defining treatment contrasts having practical meaning. Shortly, it is desirable always to find set of basic contrasts. They are useful to construct any contrasts in accordance with wishes of an experimenter. Generally, any treatment contrast is a linear combination of these basic contrasts which are estimable in the same stratum as they. They include, inter alia, any elementary comparisons of the doses of urea effects, i.e. between effects for $N_1 - N_2$, $N_1 - N_3$, $N_1 - N_4$, $N_1 - N_5$, and $N_1 - N_6$. The first elementary contrast, $N_1 - N_2$, is also the basic contrast

$\mathbf{c}_{57}'\boldsymbol{\tau}$, which analysis is given in Table 5. Let us denote the mentioned contrasts by $\mathbf{s}_1'\boldsymbol{\tau}$, $\mathbf{s}_2'\boldsymbol{\tau}$, $\mathbf{s}_3'\boldsymbol{\tau}$, $\mathbf{s}_4'\boldsymbol{\tau}$ and $\mathbf{s}_5'\boldsymbol{\tau}$, respectively. In Table 6 there are forms of them, their estimates, variance estimates, calculated values of test statistic F and corresponding p values used to test particular hypothesis connected with them (see Ambroży-Deręgowska et al. 2015).

Consider for instance contrast $\mathbf{s}_5'\boldsymbol{\tau}$ between doses N_1 and N_6. It can be written as

$$\mathbf{s}_5'\boldsymbol{\tau} = [1, 1, 1, 1]' \otimes [1, 0, 0, 0, 0, -1]' \otimes [1, 1, 1]'\boldsymbol{\tau}\,.$$

This contrast is estimable in the subplot stratum (2). From Table 3 it is easily seen that in this stratum five basic contrasts for doses of nitrogen ($\mathbf{c}_h'\boldsymbol{\tau}$, h = 57, 60, 63, 66, 69) are estimable. So we obtain

$$\mathbf{s}_5'\boldsymbol{\tau} = 1.2247 \cdot \mathbf{c}_{57}'\boldsymbol{\tau} + 0.7071 \cdot \mathbf{c}_{60}'\boldsymbol{\tau} + 0.5 \cdot \mathbf{c}_{63}'\boldsymbol{\tau} + 0.3873 \cdot \mathbf{c}_{66}'\boldsymbol{\tau} + 1.8974 \cdot \mathbf{c}_{69}'\boldsymbol{\tau}$$

It is easy to check the contrast is estimated with full efficiency $[\,\mathrm{E}_2(\mathbf{s}_5'\boldsymbol{\tau}) = 1\,]$ in the inter-subplot stratum. Taking into account the estimates $\widehat{(\mathbf{c}_h'\boldsymbol{\tau})}_2$, h = 57, 60, 63, 66, 69 (see, Table 3) the estimate of the contrast $\mathbf{s}_5'\boldsymbol{\tau}$ in the stratum (2) (for b stage_bl) is equal to

$$\begin{aligned}\widehat{(\mathbf{s}_5'\boldsymbol{\tau})}_2 &= 1.2247 \cdot (-0.0522) + 0.7071 \cdot (-0.1456) + 0.5 \cdot (-0.1413) + \\ &+ 0.3873 \cdot (-0.1923) + 1.8974 \cdot (0.1067) = -0.1096\end{aligned}$$

From Table 2 we have that variance estimate of the error in the stratum (2) is equal to $\mathrm{MSE}_2 = 0.0021$. So the estimate of the variance of the contrast is 0.0128, then

$$F = [\,\widehat{(\mathbf{s}_5'\boldsymbol{\tau})}_2\,]^2 / 0.0128 = 0.9400.$$

Table 6. Set of the elementary contrasts of type N (nitrogen) for chlorophyll content in different stages of maize variety 'Anjou 258'

Basic contrasts	Coefficients λ_{2h} for the elementary contrasts				
j	$s'_1\tau$	$s'_2\tau$	$s'_3\tau$	$s'_4\tau$	$s'_5\tau$
57	2.4495	1.2247	1.2247	1.2247	1.2247
60	0	2.1213	0.7071	0.7071	0.7071
63	0	0	2	0.5	0.5
66	0	0	0	1.9365	0.3873
69	0	0	0	0	1.8974
Chlorophyll *a* stage_56					
$\widehat{(s'\tau)}_2$	-0.851	-1.397	-3.161	-3.2179	-2.0614
Estimated variances	0.265	0.265	0.265	0.2652	0.2652
F	2.727	7.361**	37.675**	39.0398**	16.021**
p	0.104	0.009	<0.0001	<0.0001	0.0002
Chlorophyll *a* stage_bl					
$\widehat{(s'\tau)}_2$	-0.494	-2.391	-3.6649	-4.9010	-1.3217
Estimated variances	0.436	0.436	0.4360	0.4360	0.4360
F	0.559	13.116**	30.8080**	55.0942**	4.0068**
p	0.457	0.0006	<0.0001	<0.0001	0.0498
Chlorophyll *b* stage_56					
$\widehat{(s'\tau)}_2$	-0.178	-0.175	-0.389	-0.405	-0.272
Estimated variances	0.008	0.008	0.008	0.008	0.008
F	3.753	3.642	18.01**	19.452**	8.793**
p	0.0574	0.0611	0.0001	<0.0001	0.0043

Table 6. continued

Chlorophyll *b* stage_bl					
$(\widehat{\mathbf{s}'\tau})_2$	-0.128	-0.3728	-0.4496	-0.6099	-0.1096
Estimated variances	0.0128	0.0128	0.0128	0.0128	0.0128
F	1.2811	10.8753**	15.8175**	29.113**	0.9400
p	0.2622	0.0016	0.0002	<0.0001	0.3362
Chlorophyll *a*+*b* stage_56					
$(\widehat{\mathbf{s}'\tau})_2$	-1.310	-1.8817	-3.9067	-3.9568	-2.6349
Estimated variances	0.3374	0.3374	0.3374	0.3374	0.3374
F	5.0859*	10.4936**	45.2294**	46.3977**	20.5742**
p	0.0278	0.0020	<0.0001	<0.0001	<0.0001
Chlorophyll *a*+*b* stage_bl					
$(\widehat{\mathbf{s}'\tau})_2$	-0.623	-2.7681	-4.1206	-5.5191	-1.4335
Estimated variances	0.5839	0.5839	0.5839	0.5839	0.5839
F	0.6639	13.1233**	29.0801**	52.1690**	3.5194
p	0.4184	0.0006	<0.0001	<0.0001	0.0655

* p < 0.05; ** *p* < 0.01

The results presented in Table 6 indicate nonsignificant influence of the dose of nitrogen N_2 – 30 kg N ha^{-1} effect on the chlorophyll content effect in all stages of development of maize variety ‘Anjou 258’ (apart of one trait – chlorophyll *a*+*b* stage_56). But it can be seen (Table 6) that the dose of nitrogen N_3 – 60 kg N ha^{-1} effect significantly increased the mean chlorophyll content in all stages. Also an even greater increase in mean chlorophyll content was observed at doses of nitrogen N_4 – 90 and N_5 – 120 kg N ha^{-1}. These contrasts are statistically significant.

Significant increase in the mean chlorophyll content were also obtained at the dose N_6 –150 kg N ha^{-1}, for all traits apart two of them (chlorophyll *b* stage_bl and chlorophyll *a*+*b* stage_bl). It can be noticed that increase in the mean content however is less, compared with that obtained at the doses from N_3 to N_5.

Comments

There is a problem concerning the inference on the base of treatment contrasts. Which test should we use. Usually we define a few contrasts. We can infer about them individually and independently. In this case we used to apply ordinary not simultaneous test (as in Table 6). But we should remember that each contrast is to be consider independently and we cannot extent the inference to other contrasts of interest.

The wider inference we have when we use simultaneous test procedures. In the considered case of the particular analysis Dunnett test is recommended. Probabilities obtained in testing elementary contrasts by this method are presented in Table 7.

In this case we can apply a little more conservative but simpler test based on Bonferroni inequality also. In this method it is enough to divide the recommended significant level by a number of contrasts tested. In our case choosing total significant level as $\alpha = 0.05$ to test 5 particular hypotheses we have to use the significant level $\hat{\alpha} = 0.01$. Then the final $\overline{\alpha}$ significant level for all 5 hypotheses defined by elementary contrasts is equal $\overline{\alpha} =$ 0.04999. ($\overline{\alpha} = 1 - (1 - \hat{\alpha})^k$, k – the number of hypotheses).

Similarly using in Table 6 for all elementary contrasts Bonferroni method, instead of $\alpha = 0.01$ we have to use $\hat{\alpha} = 0.002$ and then $\overline{\alpha}$ = 0.00996.

Table 7. Results of Dunnett test for elementary contrasts of type N (nitrogen) for chlorophyll content in different stages of maize variety 'Anjou 258'

Nitrogen	Chlorophyll *a* stage_56 MS = 0.04421 df = 60	Chlorophyll *a* stage_bl MS = 0.07266 df = 60	Chlorophyll *b* stage_56 MS = 0.00140 df = 60	Chlorophyll *b* stage_bl MS = 0.00213 df = 60	Chlorophyll *a+b* stage_56 MS = 0.05624 df = 60	Chlorophyll *a+b* stage_bl MS = 0.09731 df = 60
	{1}	{1}	{1}	{1}	{1}	{1}
Means	1.4121	1.4896	.17383	.23350	1.5599	1.7256
N_1-0						
N_2-30	0.338341	0.912950	0.203673	0.683785	0.106378	0.882130
N_3-60	0.036173	0.002777	0.215125	0.007341	0.008694	0.002769
N_4-90	0.000000	0.000003	0.000374	0.000900	0.000000	0.000005
N_5-120	0.000000	0.000000	0.000209	0.000005	0.000000	0.000000
N_6-150	0.000828	0.179825	0.018696	0.793780	0.000134	0.228554

4. Remarks

One problem is worth considering. It is connected with meaning of years effects in a model of observations. Usually they are treated as fixed (as in the present paper) or random. None approach is not appropriate.

From randomization point of view, only the first year of an experiment is chosen randomly, and the next ones are not randomized (they are successive years). It means that randomization of years is very restricted. Therefore the natural order of years recommends to treat the year effects as fixed. Then the inference concerns only the future years having the environmental conditions as the years of the experiment performed. However, recent environmental conditions (climate mainly) are highly variable and the years of the experiment cover a wide range of potential variability of the climate and soil conditions.

Assuming the years effects as random effects in a model of observations has also weak point. Then the years of an experiment are treated as a sample from a finite or an infinite population of years. But probability of choosing three or four successive years from such population for the particular experiment is very small. It leads to a high-risk inference on the basis of a short series of experimental years about all potential years. Another question is do we need inference for infinite number of years?

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