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TWO-FACTOR AGRICULTURAL EXPERIMENT WITH REPEATED MEASURES ON ONE FACTOR IN A COMPLETE RANDOMIZED DESIGN

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ABSTRACT

A typical agricultural experiment involves comparisons of several treatments at different points in time. The ensuing lack of independence between observations of the same experimental unit may then impair the attainment of statistical significance by the standard analysis of variance, and calls for the application of more powerful methods. This paper addresses one such method, the so-called two-factor experiment with repeated measures on one factor. We discuss the adequacy of this model in the context of three concrete examples drawn from agricultural experimentation.

Keywords: Two-factor ANOVA, statistical power, multivariate analysis, repeated measures.

1. INTRODUCTION

Consider the two-factor agricultural experiment with repeated measures on one factor, such as time. The layout for this design is presented in Table 1.

For this problem, the univariate mode of analysis (ANOVA) is generally more powerful than the multivariate approach, provided the assumptions underlying the univariate model are valid and the hypothesis under consideration involves time (Arnold, 1981; Crowder and Hand, 1990; Rouanet and Lepine, 1970; Rogan, Keselman and Mendoza, 1979). Otherwise, specific cases may favor the significance of the multivariate approach, although the apparent increase in power may also indicate an increase in type I error rate. We present examples, in fact counterexamples, addressing these cases.

The statistical model for the univariate ANOVA is

$$X_{ijk} = \mu + \beta_j + \pi_{jk} + \alpha_i + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (1)$$

where μ is the mean level, the α_i are the marginal time effects across treatments, the β_j are the marginal means for the treatments

across time, the π_{jk} are the average deviations of the k th unit about the marginal mean for the j th treatment group, the $(\alpha\beta)_{ij}$ represent the interactive time dependent magnitudes of the treatment effect for the j th group at the i th time. For different k the π_{jk} are uncorrelated. For given k the correlations between the π_{jk} are constant. The ε_{ijk} are all uncorrelated and the ε_{ijk} and π_{jk} are also uncorrelated. Furthermore, the ε_{ijk} and π_{jk} are normally distributed. The validity of (1) depends on the equality of the covariance matrices of the orthonormal contrasts across treatments, and for time-related hypotheses on sphericity ($\text{Var}(X_{ijk} - X_{i'jk}) = \text{constant}$). Sphericity is usually assessed through a sphericity factor ε , a function of the elements of the common covariance matrix (Winner, 1971). If sphericity holds, ε equals 1. Otherwise, ε is smaller than 1. Sphericity can be tested with Mauchly's test (Morrison, 1976). If sphericity is rejected, then the standard F value for testing time-related hypothesis can be reduced according to the Greenhouse-Geisser or Huynh-Feldt corrections (Winer, 1971). Alternatively, for each a priori contrast of interest one can perform a separate-error F test. That is, an F test using only the covariance matrix relevant to the specific contrast (Maxwell, 1980; Boick, 1981).

In this paper the multivariate approach is based on Hotelling's T^2 or Wilk's Δ (Morrison, 1976). Provided that normality and homogeneity of the (common) covariance matrix hold, the multivariate method is always a legitimate method to adopt.

The examples that follow are based on real experiments but with some data modified to emphasize main points. All computations were performed in SAS (1988).

Example 1

Yield (kg/plot) of cherry tomatoes (*Lycopersicum esculentum* var. *cerecifforme*) from two different training systems: one-stem system vs four-stem system. The data from this experiment are presented in Table 2 and results of the statistical analyses are shown in Table 3.

Table 3 shows no significant interaction. Consider then the dates' main effect (also shown in Table 3). It is well known from the literature (see, for example, Crowder and Hand, 1990) that under H_0 and lack of sphericity the univariate test tends to reject too many H_0 's (which is not the case here). As Table 3 shows, the

Greenhouse-Geisser correction depresses the original univariate F-value, making results even less significant under the univariate approach. Therefore, under H_1 and non-sphericity the multivariate test may have greater power than the univariate method. However, suppose one is a priori interested in a contrast between the first measurement versus the fourth. It can be shown that the resulting F value for that specific contrast is statistically significant at $p < 0.02$. This result is more in line with the significance of the multivariate approach presented in the Table.

Example 2

A study was conducted to correct copper deficiency in calves. Calves were randomly assigned to one of three treatments, homeopathic copper supplement or standard commercial supplement or control. Body weights (kg) of treated calves were measured on three different occasions. The resulting data set is presented in Table 4 and the results of statistical analyses are shown in Table 5.

Table 5 points to the existence of a significant interaction ($\alpha=0.05$) via multivariate approach. Notice that a very small difference in the p value can lead to a substantially different decision. The detection of a significant interaction would call for testing the significance of the simple main effects rather than the overall main effects.

As Table 5 shows both tests have the same numerator degrees of freedom, but the univariate tests has greater denominator degrees of freedom. Under H_0 and sphericity the multivariate test generally tends to underestimate F (which is not the case here).

It is conceivable that in this particular instance Mauchly's test failed to reject a false H_0 (sphericity). Mauchly's test is not always powerful (Huynh and Mandeville, 1979). If the underlying model is not spherical relevant comments made above for example 1 would also apply here. The same is true if H_1 holds.

Example 3

An experiment was carried out to improve weight of pigs. The data set in Table 6 represents measurements (kg) on six different times. The results of statistical analyses are shown in Table 7.

The hypothesis of overall treatment differences does not involve time. Under the usual assumptions univariate and multivariate tests

are always valid, regardless of the sphericity condition (Arnold, 1981), but the multivariate method may be more powerful.

Notice that unlike time-related hypotheses, the power of this type of hypothesis under the univariate procedure decreases as intraclass correlation increases (Arnold, 1981). Therefore, under the univariate model the intraclass correlation affects the power of these two types of hypotheses in opposite directions.

2. DISCUSSION

For time-related hypotheses the univariate test averages estimated variances of all contrasts. The resulting tests on individual contrasts may then have denominators which are too small or too large. Thus the counterexamples present here.

Simulation results in the literature indicate that the magnitude of the loss in power comparing multivariate and univariate tests ranges from 5 to 11 percent if the sphericity assumption is approximately correct (Mendoza et al, 1974). However, if $\epsilon < 0.75$ the multivariate procedures are consistently more powerful than their univariate alternatives, with the loss in univariate power ranging from 7 to 21 percent. According to Rogan et al (1979) departures from the sphericity assumption cause small changes in the nominal type I errors. These authors also investigated the effect of departures from the normality assumption on type I and type II errors. It should be noted that if the distribution is normal the increase in power of the T test cannot be due to an increase of type I error, since the actual level of this test is always $\leq \alpha$ under normality (Morrison, 1976). However, if the distribution is not normal, results from these simulation studies show that the increase in power of multivariate procedures may also indicate an increase in the type I error ranging from 0.045 to 0.059 for a nominal 0.05 level.

Crowder and Hand (1990) suggest that if one has strong prior reasons for believing that sphericity holds then one should perform the standard unmodified F-test, and if not then one should perform either a modified F-test or a multivariate test.

Our suggestion stems from the ideas of Rouanet and Lepine (1970) and Boick (1981): If possible, perform the multivariate test (testing interactions perhaps at 10%). If results are significant then stop. If results are not significant, perform Mauchly's test and estimate the covariance matrix of contrasts. Examine all relevant

facts. Adapt the univariate test. Use error term appropriate for each contrast.

3. SUMMARY

We have discussed the two-factor experiment with repeated measures on one factor. We introduced three counterexamples involving higher actual significance for the multivariate testing. We have proposed an approach stemming from Boick's work on separate F tests. We conclude that no automatic procedure is likely to produce the right choice of testing approach. Rather, all relevant facts, univariate, multivariate and Mauchly's test must be taken into account.

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Table 1. Layout of two-factor ANOVA with repeated measures on one factor

Time	Treatment 1			Treatment 2			...
	Units			Units			...
	1	2	...	1	2	...	
1							
2							
...							

Table 2: Yield (kg/plot) cherry tomatoes from two different training systems on different dates (Example 1)

D	Treat. 1							Treat. 2						
	U1	U2	U3	U4	U5	U6	U7	U1	U2	U3	U4	U5	U6	U7
1	1.896	1.545	1.955	1.662	1.721	2.072	1.545	2.130	1.311	2.130	1.838	1.779	0.960	1.253
2	2.072	1.545	2.013	1.896	1.779	1.838	1.545	1.955	1.662	2.130	1.896	1.955	0.902	1.253
3	1.896	1.779	2.130	1.838	1.779	0.609	1.253	1.779	1.545	2.130	1.896	1.896	0.609	1.838
4	2.364	1.838	2.130	1.721	2.013	1.896	2.013	2.247	1.545	2.364	2.072	1.662	0.785	1.838
Av	2.057	1.677	2.057	1.779	1.823	1.604	1.589	2.028	1.516	2.189	1.926	1.823	0.814	1.546
	Av: 1.799							Av: 1.691						

D: date: 1: 05-01-92, 2: 05-15-92, 3: 05-30-92, 4: 06-01-92. Treat.1: one-stem system treatment. Treat.2: four-stem system treatment. Av: Arithmetic average. Ui: experimental unit.

Table 3. Results from example 1

Effect	Method	Num.df	Den.df	F	P
Treatment by date interaction	Univariate	3	36	0.87 *	0.44
	Multivariate	3	10	0.87	0.49
Dates	Univariate	3	36	2.66 *	0.10
	Multivariate	3	10	7.62	0.01

Num.df, Den.df: numerator and denominator degrees of freedom, respectively.
 * Greenhouse-Geisser corrected F statistic. Mauchly's test: p < 0.001.

Table 4. Data from trial on the incidence of copper in calves' weight (Example 2)

O	C					S					H				
	U1	U2	U3	U4	U5	U1	U2	U3	U4	U5	U1	U2	U3	U4	U5
1	50	90	75	60	59	77	65	78	60	85	93	56	60	98	80
2	59	112	84	76	69	94	87	92	78	96	120.3	75.2	81.2	119.3	106.3
3	87	135	106	100	94	115	116	118	104	120	142.6	97.7	92.7	147.6	125.6
Av	65.3	112.3	88.3	78.7	74	95.3	89.3	96	80.7	100.3	118.6	76.3	78	121.6	104
	Av: 83.7					Av: 92.3					Av: 99.7				

O: occasions: 1: 11-27-91, 2: 12-26-91, 3: 02-01-92. C: Control. S: Standard commercial supplement. H: Homeopathic supplement. Av: Arithmetic average. Ui: Experimental unit.

Table 5. Results from example 2

Effect	Method	Num.df	Den.df	F	p
Treatment by occasion interaction	Univariate	4	24	2.69	0.06
	Multivariate	4	22	2.96	0.04

Num.df, Den.df: numerator and denominator degrees of freedom, respectively. Mauchly's test: $p = 0.18$

Table 6. Weight of pigs (kg) on six occasions (Example 3)

T	Treat. 1					Treat. 2					Treat. 3				
	U1	U2	U3	U4	U5	U1	U2	U3	U4	U5	U1	U2	U3	U4	U5
1	40.6	44.3	37.5	49.9	48.3	58.8	36.0	53.0	60.7	55.4	53.3	53.9	47.7	68.4	45.9
2	42.2	74.6	63.8	67.5	54.5	73.0	48.3	76.1	82.3	71.5	73.0	66.9	57.6	74.6	53.9
3	57.6	88.5	79.2	83.5	69.9	74.6	65.6	75.8	88.5	82.6	92.2	82.0	75.5	79.2	66.9
4	55.7	84.1	84.5	80.1	63.1	61.9	49.6	80.8	96.8	86.9	92.2	72.1	71.5	85.7	61.9
5	34.7	67.5	79.8	88.8	73.6	70.6	75.2	78.0	107.3	100.5	95.3	75.5	78.0	95.6	64.4
6	44.0	81.4	91.2	89.1	78.0	84.5	72.7	109.2	116.9	108.5	107.0	88.2	86.9	100.5	80.1
Av	45.8	73.4	72.7	76.5	64.6	70.6	57.9	78.8	92.1	84.2	85.5	73.1	69.5	84.0	62.2
	Av: 66.6					Av: 76.7					Av: 74.9				

T: Times: 1: 10-01-93; 2: 10-15-93; 3: 10-25-93; 4: 10-30-93; 5: 11-05-93; 6: 11-15-93. Treat.1: Treatment 1. Treat.2: Treatment 2. Treat.3: Treatment 3. Av: Arithmetic average. Ui: experimental unit.

Table 7. Results from example 3

Effect	Method	Num.df	Den.df	F	p
Treatment by date interaction	Univariate	10	60	1.71 *	0.16
	Multivariate	10	16	2.14	0.08
Treatment	Univariate	2	12	1.03	0.39
	Multivariate	12	14	2.56	0.048

Num.df, Den.df: numerator and denominator degrees of freedom, respectively.

* Greenhouse-Geisser corrected F statistic. Mauchly's test: $p = 0.009$.