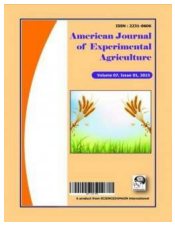

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Statistical Analysis for Correlated Paired-plot Designs

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DP, CAMA and ARS designed the field experiment which motivated theoretical statistical developing. Author DP wrote the first draft of the manuscript. Authors DP and GMF reviewed all drafts of the manuscript and managed and performed the statistical analysis and simulation study of the work. All authors read and approved the final manuscript.

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ABSTRACT

This work is aimed to evaluate different statistical analysis with a randomized complete block design for paired-plot studies of herbicide selectivity in sugarcane experiments. Two procedures were considered: i) the construction of a t-test to assess the hypothesis of the paired-plot mean difference to be zero in each treatment; or ii) the use of an analysis of variance where the treated and paired plots were considered in a split-plot model and the interaction is sliced by treatment. By simulation with normal bivariate distributions, with uniform (zero, 0.11, 0.33, 0.44, 0.67 or 0.89) or

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heterogeneous correlations, the two procedures showed similar performance. The power of the tests increases as the correlation of paired-plot increases.

Keywords: Selectivity; herbicides; sugarcane; multiple comparisons; simulation.

1. INTRODUCTION

Classic experiments are conducted in various areas that allow assessing responses in paired comparisons. For example, observing an attribute of the sampled plot before and after a treatment or observing responses to similar parts treated and untreated. The performance of paired comparisons can be more interesting and more realistic to assess the ordering of treatments (or processes of interest) than the simple ordering of responses directly [1-2].

The example discussed in this study was motivated by assessments studies on selectivity of herbicides in sugarcane crops, but it can also be applied to other studies on pests, diseases, weeds, etc. It is also applicable when measurements of the attribute, whether visual or mechanical, need to be calibrated by non-treated plot, preferentially to the side, or with repeated readings after a reference measurement.

For the specific case that motivated this research, consider evaluating the selectivity of five treatments (herbicides) in a sugarcane cultivar and the use of four replications in a randomized complete block design. The allocation can be made with five plots per block, each divided into two plots, applying herbicide in half and leaving the other half as paired control [3] or each block can be formed with 10 plots with allocation in pairs, applying herbicide in one and leaving the other as paired control. Azania et al. [4] showed an analysis where paired-plots were allocated as a supplementary treatment called control. If this method were applied to the example in question, each of the five treatments would have four replicates and control (treatment 6) with 20 replications. The proposed analysis compares the design with the classic case of just one control per plot, but it does not consider the paired comparison that actually exists. There are also other parametric or non-parametric forms to analyze paired-plot experiments [1-2].

This study discussed two forms of statistical analysis for these designs, as well as the effects of different correlations between paired comparisons.

2. MATERIALS AND METHODS

2.1 Simple Analysis of Variance per Condition

For an example with five pairs of plots in each of four replications in a randomized complete block design, the analysis of variance scheme, per condition, is that presented in Table 1. A multiple comparisons test can be used with significant F-value ($p < 0.05$) [5]. Among the alternatives, probably the most interesting for biological fields is the usual t test (LSD = least significance difference), whose minimal significant difference is given by LSD = t x (Mean Square of Error/rep)$^{1/2}$. In this case, we have t = 2.1788 (tabled value, Degree of Freedom (DF) = 12, $p<0.05$) and rep = 4 replications. When there are many treatments, an interesting alternative to control false significance would be False Discovery Rate (FDR) procedures [6] rather than conservative procedures such as Tukey [7] or Scott and Knott [8].

2.2 Analysis of Mean Difference between Treated and Paired Plots

Working with differences (T-P), the analysis in Table 1 allows to accept whether the means of differences (T-P) in each treatment are equal or, using a test for multiple comparisons, which are different.

In practice, however, the interest can be a test for the hypothesis of the mean differences (T-P) in each treatment be zero. Rejecting this hypothesis indicates the treatment effect. The test statistics is for each pairing: TC = dif(T-P)/ [t x (Mean Square of Error/rep) $^{1/2}$]. In bilateral manner, we have t = 2.1788 (tabled value, p = 0.05) and rep = 4 (replications). Using this t value in the previous formula, we can have the minimum value significant to differ from zero (in absolute value): dif (T-P) = 0 = LSD (0) = | t x (Mean Square of Error/rep) $^{1/2}$ |.

In the example, the DF of the residual is 12, resulting t = 2.1788 (tabled value, p = 0.05). For experiments with DF greater than 20, t = 2 can be used, simplifying the formula: LSD(0) = | 2 x (Mean Square of Error/rep) $^{1/2}$ |.

The LSD of the previous item, as well as LSD (0) of this item may present low level of significance set per experiment [5], especially for many treatments, but it is good not to greatly increase type II error (fail to detect differences when they exist), according to [9].

2.3 Analysis of Variance Using the Split-plot Model

For this analysis (Table 2), each pair of plots (treated and paired) are considered experimental plots.

In this case, F2 statistics (Table 2) tests the overall effect of the pairing, that is, if the overall mean of the treated plots is equal to the control mean, and F3 statistics (Table 2) tests the interaction treatments x pairing. If it is significant, it is interesting to evaluate the pairing by treatment (Table 3), which is another test for the difference equal to zero in each pairing.

2.4 Comparison between Statistics of Items 2.2 and 2.3

There are statistical differences in the construction of the tests. In the TC case (item 2.2), we use the differences, pair-to-pair, and the respective residual variance of these differences. In the split-plot design, responses of treated and non-treated are used, building up contrasts between pairing means. There are differences in residual variances, depending on the correlation or covariance between the paired-plots and associated DF (12, in example in Table 1) of TC statistics of item 2.2 and 15 (Table 2) in F statistics of item 2.3. To compare the statistics of the procedures, 1000 trials (replicas) are simulated in each situation.

Table 1. Scheme of analysis of variance per condition

Sources of variation	Degrees of freedom (DF)	Mean squares	F statistics
Block	3	MSB	
Treatment	4	MSH	MSH/MSError
Residual	12	MSError	

To focus on specific cases, normal bivariate distributions are considered: Case (1) with uniform correlations in all treatments, evaluating increasing values, namely: 0.00 (independent), 0.11, 0.33, 0.44, 0.67, 0.89 and Case (2) heterogeneous correlations (different combinations from the above) according to replications (in this case, blocks) of each of the treatments.

Table 2. Scheme of analysis of variance of split-plot model

Sources of variation	DF	Mean squares	F statistics
Block	3	(1)MSB	
Treatment	4	(2)MSH	F1=(2)/(3)
Residual (a)	12	(3)MSError (a)	
General pairing	1	(4)MSPair	F2=(4)/(6)
Paring x Treatment	4	(5)MS(Pair x Treat)	F3=(5)/(6)
Residual (b)	15	(6)MSError (b)	

In the first situation, to estimate the power of the test (ability to detect differences) using the means of treatments (treated and paired, in Table 4), uniform standard deviation 4.5 for both treated and paired, compatible covariances with stipulated correlations (Cases 1 and 2, described in the previous paragraph) and fixed effects of blocks (-10, -5, 5, 10) are used. In a second situation, to estimate the rate of type I error (rejecting equalities, when they exist), the treated and paired were equal (using A = 130, B = 110, C = 120, D = 120, E = 120), effects of covariance and blocks, as in previous cases.

Table 3. Scheme of analysis of variance of paired plots per treatment

Sources of variation	DF	Mean squares	F statistics
Paired-plots in A	1	(7) MSPairA	F2 A=(7)/(6)
Paired-plots in B	1	(8) MSPairB	F2 B=(8)/(6)
Paired-plots in C	1	(9) MSPairC	F2 C=(9)/(6)
Paired-plots in D	1	(10) MSPairD	F2 D=(10)/(6)
Paired-plots in E	1	(11) MSPairE	F2 E=(11)/(6)

For each simulated trial, 1000 replicas of the two analyses are performed, described in items 2.1 to 2.3. With TC (item 2.2) and F (item 2.3, Table 3) statistics, p-values were obtained the (probability of rejection of no difference between treated and paired). The simulation study was performed in the computing environment R, version 3.1.1 [10]. It was used in the R environment, besides the basic distribution packages, the package “MASS” [11] to generate

bivariate normal distributions by means of the function “mvrnorm”. Further information on the use of the R environment can be found at Matloff [12].

3. RESULTS AND DISCUSSION

For one of the simulations of responses for sugarcane production (tons per hectare), the results could be those presented in Table 4. Treatment A increases the mean response of 15.00 (ton. ha^{-1}), treatment B decreases the same 15.00 (ton. ha^{-1}), C and D decrease 9.00 (ton. ha^{-1}) and 10.50 (ton. ha^{-1}) and E increases only 1.50 (ton. ha^{-1}), that is, a situation with different effects of treatments is simulated to evaluate the power of the test.

Table 4. Means of simulation (ton.ha^{-1}) under five treatments in treated plots and paired control (4 replications)

Treatments	Treated (t)	Paired (p)	Differences (t-p)
A	135.00a	120.00a	15.00a
B	112.75b	127.75a	-15.00c
C	117.50b	126.50a	-9.00c
D	115.25b	125.75a	-10.50c
E	121.75ab	120.25a	1.50b
MSError	74.86	40.99	45.07
LSD	13.33	9.86	10.34

Note: same lowercase letters in each column, equal treatments (p > 0.05, LSD)

3.1 Simple Analysis of Variance per Condition

With this analysis, we observe among the treated (T), treatment A shows the greatest response. Paired control (P) are equal (p > 0.05) and the differences (T-P) show treatment A with the highest positive difference, differing from treatment E, which differed from other three showing negative differences and are similar (same letter c).

3.2 Analysis of the Mean Difference between Treated and Paired Plots

For a hypothesis test of difference (T-P) to be zero, with the data of the example, we have: dif(T-P)=0= LSD(0)=| t x (Mean Square of Error/rep) $^{1/2}$ |= | 2.1788 x (45.07/4) $^{1/2}$ |=7.31.

Thus, the differences (T-P) can be accepted as zero for treatment E, positive for A (treatment increasing the response) and negative for treatments B, C and D (treatments decreasing the response). This type of analysis responds easily to the immediate interest of the researcher. In addition, the block effect explicit in item 2.2 model can be used to accommodate extra interference source during the assessment or management of the experiment, conducting operations per blocks (practical interest).

3.3 Analysis of Variance Using the Split-plot

For this analysis, the plots of each pair (treated and paired) are considered experimental plots. F3 statistics, calculated as shown in Table 2, indicates that the significance of pairing x treatment interaction and pairing per treatment was evaluated (Table 5). Pairing in E (p > F = 0.7418) suggests the absence of effects of this treatment. For the other treatments, values (p > F) suggests effects of treatments, although for treatment C, unlike LSD (0), the value (p > F) is slightly higher than 0.05. The reasons for these differences are discussed in items 2.4 and 3.4.

Table 5. Assessment of paired-plots per treatment

Sources of variation	DF	Mean squares	F statistics	p-value (p > F)
Paired-plots in A	1	450.00	11.27	0.0043
Paired-plots in B	1	450.00	11.27	0.0043
Paired-plots in C	1	162.00	4.06	0.0623
Paired-plots in D	1	220.50	5.52	0.0329
Paired-plots in E	1	4.50	0.11	0.7418
Residual (b)	15	39.93		

3.4 Comparison between Statistics of Items 2.2 and 2.3

To summarize the simulation results, Tables 6 and 7 show the means and standard deviations of p-values of TC (item 2.2) and F (item 2.3, Table 3) statistics, as well as the frequencies of p-values that exceed 0.05 (rejection of no treatment effect).

In the case of differences (T-P), the power of the test is similar for the two procedures, in all treatments (Table 6). In treatment E, with the smallest difference (T-P), the difference increases with increasing correlation between paired plots, in other words, the increase of this correlation makes detection of the paired

treatment effect easier. The same fact also occurs for the other treatments with greater difference (T-P), however, it is not observed in Table 6, because the power of the test proved to be virtually 100%. In the case of heterogeneous covariance, the power of the tests was similar to that of the covariance mean (or correlation). In summary, for all cases, frequencies of hypothesis rejection of zero differences are similar in both procedures, TC (item 2.2) and F (item 2.3, Table 3) statistics. The presence of positive correlation between paired plots may occur in practice, mainly through the spatial proximity of adjacent plots, and it makes detection of the paired treatment effect easier, mainly if the difference (T-P) is smaller, which is desirable for the experimental standpoint.

With respect to type I errors (Table 7), the two procedures are similar with means (M) of p-values around expected (0.5) and the same occurs for rejection frequency of the hypothesis of no difference between means of treated and paired plots (around 50 in 1000). The standard deviations (SD) of p-values are similar to the two statistics, in Tables 6 and 7.

Table 6. Means (M) and Standard Deviations (SD) of p-values and frequencies of (p<0.05) with two statistics in 1,000 simulated replicas in each paired-plot design (power of test), for treatments in Table 4, using normal bivariate with different correlations. Detail for Treatment E

Treatment	TC statistics (item 2.2)			F statistics (item 2.3)		
	M	SD	p < 0.05	M	SD	p < 0.05
Case 1 - COV_UNI_1 (cov = 0, correlation between paired plots=0.00)						
E	0.3628	0.2976	169	0.3706	0.3011	159
Case 1 - COV_UNI_2 (cov = 0.5, correlation between paired plots =0.11)						
E	0.3568	0.2943	173	0.3559	0.2919	167
Case 1 - COV_UNI_3 (cov = 1.5, correlation between paired plots =0.33)						
E	0.3028	0.2816	204	0.3287	0.2964	214
Case 1 - COV_UNI_4 (cov = 2, correlation between paired plots =0.44)						
E	0.2996	0.2858	223	0.2990	0.2804	220
Case 1 - COV_UNI_5 (cov = 3, correlation between paired plots =0.67)						
E	0.1965	0.2337	371	0.2170	0.2542	364
Case 1 - COV_UNI_6 (cov = 4, correlation between paired plots =0.89)						
E	0.0435	0.0988	795	0.0384	0.0891	821
Case 2 – heterogeneity of covariance within each block						
E	0.2711	0.2672	229	0.2849	0.2782	222

Other treatments: Cases 1 and 2.						
Treatment	**TC statistics (item 2.2)**			**F statistics (item 2.3)**		
	M	**SD**	**p < 0.05**	**M**	**SD**	**p < 0.05**
A	0.0000	0.0000	1000	0.0000	0.0000	1000
B	0.0000	0.0000	1000	0.0000	0.0000	1000
C	0.0000	0.0000	1000	0.0000	0.0000	1000
D	0.0000	0.0000	1000	0.0000	0.0000	1000

Table 7. Means (M) and Standard Deviations (SD) of p-values and frequencies of (p<0.05) with two statistics in 1,000 simulated replicas in each paired-plot with equal means between treated and paired plots (type I error of tests) using normal bivariate

COV_UNI_1 (cov = 0, correlation between paired plots =0)						
Treatment	**TC statistics (item 2.2)**			**F statistics (item 2.3)**		
	M	**SD**	**p < 0.05**	**M**	**SD**	**p < 0.05**
A	0.4759	0.2856	51	0.4900	0.2890	46
B	0.4925	0.2888	56	0.4980	0.2919	50
C	0.5180	0.2847	46	0.4838	0.2844	41
D	0.5034	0.2809	42	0.4820	0.2931	52
E	0.4862	0.2910	46	0.5081	0.2895	55
COV_UNI_2 (cov = 0.5, correlation between paired plots =0.11)						
A	0.4947	0.2770	44	0.4968	0.2851	53
B	0.4834	0.2844	59	0.5042	0.2836	50
C	0.5007	0.2887	43	0.4983	0.2889	50
D	0.4938	0.2882	52	0.4881	0.2953	51
E	0.4991	0.2929	51	0.5057	0.2814	41

COV_UNI_3 (cov = 1.5, correlation between paired plots =0.33)						
A	0.5104	0.2923	52	0.5123	0.2940	50
B	0.4944	0.2895	48	0.5141	0.2846	37
C	0.5062	0.2893	56	0.5090	0.2902	58
D	0.5029	0.2968	57	0.4930	0.2867	51
E	0.5012	0.2928	53	0.4936	0.2929	59
COV_UNI_4 (cov = 2, correlation between paired plots =0.44)						
Treatment	**TC statistics (item 2.2)**			**F statistics (item 2.3)**		
	M	**SD**	**p < 0.05**	**M**	**SD**	**p < 0.05**
A	0.4872	0.2907	57	0.5052	0.2820	51
B	0.5062	0.2843	31	0.4998	0.2886	43
C	0.4847	0.2836	47	0.5025	0.2908	46
D	0.4990	0.2856	44	0.4984	0.2901	55
E	0.5177	0.2790	33	0.5039	0.2917	47
COV_UNI_5 (cov = 3, correlation between paired plots =0.67)						
A	0.5029	0.2867	53	0.4815	0.2915	59
B	0.5141	0.2862	49	0.5114	0.2858	59
C	0.5036	0.2951	49	0.5048	0.2916	53
D	0.5086	0.2808	43	0.5024	0.2910	52
E	0.5069	0.2843	47	0.4781	0.2873	51
COV_UNI_6 (cov = 4, correlation between paired plots =0.89)						
A	0.5005	0.2896	45	0.5005	0.2893	59
B	0.4893	0.2895	50	0.5016	0.2895	56
C	0.5062	0.2801	43	0.4942	0.2931	49
D	0.4889	0.2882	54	0.5049	0.2904	51
E	0.5076	0.2889	45	0.5030	0.2911	49

Case 2 – heterogeneity of covariance within each block						
treatment	**TC statistics (item 2.2)**			**F statistics (item 2.3)**		
	M	**SD**	**p < 0.05**	**M**	**SD**	**p < 0.05**
A	0.5182	0.2803	32	0.5124	0.2813	37
B	0.5071	0.2850	42	0.4926	0.2900	55
C	0.4910	0.2932	54	0.4711	0.2895	51
D	0.4815	0.2937	62	0.4600	0.2958	73
E	0.5337	0.2755	19	0.5301	0.2808	36

4. CONCLUSION

The two procedures show similar performance for the evaluation of experiments with paired plots: i) a t test to test whether, in each treatment, the means of paired differences can be zero; or ii) an analysis of variance in which treated and paired plots are considered in a split-plot model and the unfolding of the interaction for treatment is done. With increasing correlation between paired plots, the power of tests to detect differences actually existing increases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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