

Advancing Understanding and Application of Mixed Models in Field Experiments Using Statistical Analytical Software (SAS® OnDemand for Academics): A Conceptual Analysis

Amos Cyrus¹, Alex Amos², Adam Ahmed³, Amos Alvan⁴, Florence Algaita Alvan⁵

^{1,3}Senior Scientific Officer, Raw Materials Research and Development Council, Abuja, Nigeria

²Research Fellow, Federal Institute of Industrial Research, Oshodi, Lagos

⁴Department of Biochemistry, Kaduna State University

⁵Kaduna State College of Education, Gidan Waya

Email address: ¹amoscyruskin@rmrdc.gov.ng

Abstract—Inferential statistics remains a fundamental tool in life sciences and agricultural research. Nevertheless, graduate students, postgraduate scholars, and researchers frequently encounter challenges related to core concepts and procedures, particularly in minimizing Type I and Type II errors. This paper seeks to bridge the gap between the theoretical development of mixed-model methodology and its practical application in field experimentation. It is important to note that the paper provides practical illustrations using SAS® OnDemand for Academics, a free, cloud-based version of SAS® designed for browser-based learning and instruction. This platform is especially suitable for students, universities, and independent learners interested in statistics, data analysis, and programming. While the examples are presented using this environment, comparable analytical procedures can also be implemented in other statistical software packages, such as R and ASReml. This treatise highlights key features and properties of mixed-model analysis, emphasizing its flexibility and robustness compared with traditional approaches based on ANOVA or GLM frameworks. By accommodating complex experimental structures and variance components, mixed models facilitate more reliable inference and more precise conclusions in hypothesis testing, including means separation procedures such as LSD, Duncan, and Dunnett tests. The motivation for this article stems from the limited treatment of mixed-model analysis in classroom settings, and the insufficient appreciation of its distinctions from ANOVA or GLM approaches, and the unfamiliar structure of mixed-model output relative to conventional ANOVA tables. Promoting the appropriate use of PROC MIXED not only ensures valid analysis and sound interpretation of experimental data but also provides a critical foundation for understanding advanced SAS procedures such as PROC GLIMMIX and PROC NLMIXED, which extend mixed-model methodology to data exhibiting non-normal distributions.

Keywords—Mixed Model, PROC GLM, Random Error, SAS® Software.

I. INTRODUCTION

Contemporary statistics and its application to Field Experiments could be traced back to about 90 years when a British statistician Ronald A. Fisher was contracted in 1919 by Rothamsted Experimental Station to examine field experiments accumulated since 1843 (University of Adelaide Digital Library 2006). It was the study of these experiment that made Fisher to have the conception of a technique called the Analysis of Variance, (ANOVA) and experimental design. These techniques have clarified field and laboratory experimentation in modern biological sciences, providing ways to reduce or avoid bias, harness precision and attain valid conclusions. While these conventional techniques remain widely used, new statistical methods that utilize increased computing power have positively impacted biological research.

The development and implementation of general linear models (GLM) and mixed models (MIXED) are two major advancements that have had a great impact on biological related research and other scientific disciplines since the 1970s (Searle et al. 2015).

The result of treatments in an experiment is influenced by two factors. First, the experimental materials (treatments) are

often chosen for answering a research question. Second, many factors are not of importance to the objectives of the study which but cause extraneous differences (known as experimental errors), which tend to mask the effects of the treatment. The experimental errors are either due to genetic variability in experimental materials and across growing seasons and soils or to lack of uniformity in the physical conduct of the experiment. Many experimental designs are available in the literature for minimizing or controlling the experimental errors.

The presence of the dual kinds of factors in most experiments has posed the need for determining whether or not a factor should be considered a fixed or random effect. Numerous agronomy experiments are carried out using a complete or incomplete block design with a multi-locational trial over several years. These multi-environmental experiments are needed to infer the treatment performance for future years over a wide region. In such experiments, treatments are fixed effects as reasoned above, but the extraneous factors may be fixed or random, depending on whether or not they are subject to randomization. Over the past three decades, the Mixed-model methodology has undergone significant development, initially in the context of animal breeding (Henderson, 1984) and subsequently across

various other disciplines (McLean et al., 1991; Searle et al., 1992; Verbeke & Molenberghs, 2000; Demidenko, 2019). Modern statistical software, such as the SAS[†] system, facilitates straightforward implementation of data analyses using mixed models (Littell et al., 2006; 2002). Nonetheless, despite the prevalence of both fixed and random effects in most field experiments, mixed models remain comparatively underutilized in biological and field experimental research, despite the fact that most experiments involve both fixed and random effects.

Yang (2008) identified several potential factors contributing to the underutilization of mixed models, including: (i) limited coverage of mixed-model analysis in widely used textbooks e.g. steel et al, 1997 and in classroom instruction, (ii) insufficient understanding of the distinctions between mixed-model analyses and traditional ANOVA or GLM approaches, and (iii) the unfamiliar format of outputs from mixed-model software compared to conventional ANOVA tables.

It is worth noting that this paper uses examples specific to the statistical software SAS[®] OnDemand for Academics, 2026. However, several other robust packages such as ASREML, GENSTAT, R, S-PLUS, and SPSS are also available for mixed-model analysis. Although the underlying principles are the same across these platforms, we will demonstrate mixed-model analysis using SAS[®] OnDemand for Academics, versatile software widely used by biological scientists and crop researchers. In particular, this discussion will focus on a comparative evaluation of SAS[®] PROC GLM versus SAS PROC MIXED, following an approach similar to that of Hamer and Simpson (2000).

For a more comprehensive comparison, see Littell et al. (2006).

II. JUSTIFICATION FOR MIXED MODEL ANALYSES

ANOVA and GLM users have frequently asked the imperative of MIXED MODEL analyses. It's therefore important to address this concern before continuing with a comparative assessment of the GLM and MIXED methodology. Several reasons justify the choice of mixed model analysis. The main reason is to prevent the repercussions associated with using inappropriate models and statistical tests. Although it is seldom recognized or explicitly acknowledged, many commonly used experimental designs in field trials incorporate both fixed and random effects. These designs include randomized complete and incomplete block designs, nested designs, split plot designs, and repeated measures designs. PROC MIXED is well suited for analyzing such designs and yields valid results, including accurate standard errors of differences among treatment means. In contrast, applying PROC GLM to these designs can often lead to incorrect outcomes. For instance, GLM may produce an incorrect denominator for the F test and inaccurate standard errors of treatment effects (Littell et al., 2006), particularly when additional random effects or heterogeneous error variances are present.

A major obstacle to the proper application and accurate interpretation of mixed models is the limited understanding or appreciation of the features and capabilities they provide.

Although this paper does not aim to present an exhaustive discussion of all these features, it is essential to clarify how PROC MIXED differs from PROC GLM in addressing the distinction between fixed and random effects.

III. HOW CAN YOU DETERMINE WHETHER A FACTOR IS FIXED OR RANDOM?

You may sometimes be told directly whether an effect is fixed or random. However, if you need to determine it yourself, begin by assuming it is fixed. Fixed effects are the most common and are generally what researchers are accustomed to working with. An effect is considered random when you are not primarily interested in the specific levels selected for the study. Instead, your goal is to generalize from the randomly chosen levels to a broader population of possible levels (Smith et al., 2005)

For example, consider subjects. You are not usually interested in how specific individuals such as Ivan, Penelope, Herb, or Wilhelmina perform on a task. Rather, you want to generalize their performance to people with similar characteristics. The same reasoning applies to groups. You typically do not care about the exact classroom, therapy group, or set of roommates included in the study. Instead, you want your findings to extend to other comparable groups.

There are additional cases that are less common. For instance, in a reading assessment, you are not concerned with how well children read the particular fifty words included in the test. Instead, those words are intended to represent reading ability more broadly, making words a random effect. Similarly, if food samples are taken from a few restaurant locations, the goal is not to evaluate those specific outlets but to generalize to all outlets of that chain, making locations a random effect.

On the other hand, some variables clearly should not be treated as random. Gender, for example, would not be random because the categories such as male and female are the only levels of interest, and there are no additional levels to generalize to. Likewise, drug dosage is typically a fixed effect. If you choose 10, 50, and 100 mg, it is because you want to examine the effects of those exact amounts, not to generalize to other untested dosages such as 200 mg or 500 mg. are the

Or levels included in your study are too few (<10) may yield unreliable variance estimates only ones about which you intend to draw conclusions, the effect is fixed. If the levels are randomly selected in order to make broader generalizations beyond them, the effect is random.

PROC GLM inherently treats all effects as fixed, and its use can therefore lead to incorrect F tests and inaccurate standard errors when one or more factors are actually random. These problems persist even when random effects are specified in the RANDOM statement of PROC GLM.

In contrast, PROC MIXED requires a clear and explicit specification of which effects are fixed and which are random, and this distinction is essential for valid mixed model analysis. Unfortunately, the difference between fixed and random effects is often only briefly addressed in statistics courses and textbooks. As a result, the lack of a clear distinction between fixed and random effects has generated substantial confusion and uncertainty in mixed model analysis.

In crop trials, cultivar effects are usually treated as random because selection focuses on rankings rather than direct comparisons, and years and their interactions with genotypes are generally random. The status of locations is debated: while some effects are “fixed” (e.g., soil type, long-term climate), crop improvement programs aim to predict performance across many untested locations, making it appropriate to treat location effects and their interactions as random. Demidenko, et al., 2019.

Baker (2014) proposed a two-step approach for distinguishing fixed and random factors: (1) initially declare a

factor random, then ask if its levels could repeat in the future and whether it is reasonable to choose the same levels; if yes, the factor can be fixed. (2) Adjust the declaration for statistical reasons, factors with many levels and no structure may be better treated as random using BLUP, which shrinks extreme means toward the grand mean, sometimes altering conclusions (Yang, 2007). Conversely, if factors are often treated as fixed. While in practice, this approach can create confusion about a factor’s true nature (Verbeke, G. and Molenberghs, G. 2020; Stroup et al., 2017).

TABLE 1. A Case Study of Mixed-Model Analysis Vs General Linear Model in a Randomized Complete Block Design Experiments (Please find attached the raw dataset and the SAS® software’s Output in Appendix 1)

Features/Output	PROC GLM	PROC MIXED	Explanation/Significance
Blocks (Random/Fixed)	Fixed	Random	PROC GLM treats blocks as fixed; PROC MIXED treats blocks as random, allowing inference to a broader population of blocks. (please note that random statement can be assign to any factor of interest in the analysis)
Sum of Squares for Irrigation	37,813	37,813	The Same as fixed effect test
Sum of Squares for blocks	368,377	368,378	The Same numerical effect
F-value for irrigation	6.06	6.06	confirms significant effect of irrigation
p-value for Irrigation	0.0039	0.0039	Identical; both detect significance.
Variance Components	Not estimated	Block variance = 12,565; Residual = 2,081.39	PROC MIXED provides estimates of random effects, which GLM cannot.
Least Squares Means (LSMEAN)	Basin: 290.38, Flood: 229.63, Spray: 223.75, Trickle: 299.63	Basin: 290.37, Flood: 229.62, Spray: 223.75, Trickle: 300.62	Means are almost identical; due to balanced data. It will not be the same for unbalance data
Standard Error of LS Means	20.50	42.78	MIXED SE larger because it includes block variance; GLM SE only uses residual error
Pairwise Differences (Example)	Basin–Flood: 60.75 (SE 22.81)	Basin–Flood: 60.75 (SE 22.81)	Similar. But may vary slightly with unbalanced data.
Fit Statistics (AIC/BIC)	Not provided	AIC = 328.3, BIC = 328.5	PROC MIXED provides model fit statistics for evaluating adequacy of model.

From the result of the SAS® output in TABLE 1, above, the randomized complete block design (RCBD) dataset, PROC GLM and PROC MIXED offer distinct approaches with important implications for inference and interpretation.

The main disparity between PROC GLM and PROC MIXED is depicted in the treatment of blocks and the variance component estimate. PROC GLM treats blocks as fixed effects, which shows that inference is strictly limited to the blocks included in the experiment. In contrast, PROC MIXED treats blocks as random effects, allowing inferences to be generalized to a larger population of blocks. This distinction is reflected in the estimation of variance components: PROC MIXED partitions total variability into block variance (12,565) and residual variance (2,081.39), whereas PROC GLM fails to provide these estimates.

The least squares mean (LSMEANS) proved to be identical between the two methodologies (e.g., Basin: 290.38 in GLM vs. 290.37 in MIXED), which confirms the balanced nature of the dataset. However, the standard errors of the means were slightly higher in PROC MIXED (42.78) compared with PROC GLM (20.50). This trend arises due to the fact that PROC MIXED incorporates both the block variance and the residual variance, consequently, reflecting the broader uncertainty associated with random blocks, while the pairwise differences in treatment means were similar (e.g., Basin–Flood difference = 60.75 in both procedures), their interpretation

must account for the larger standard errors in MIXED, especially in the presence of unbalanced data or missing observations.

In addition, PROC MIXED provided a model fit statistics such as AIC and BIC (328.3 and 328.5, respectively), which allow researchers to evaluate the assumptions of normality. PROC GLM lacks such capabilities for random effects, limiting its utility when assumption checking is necessary.

IV. CONCLUSIONS

The use of PROC GLM of the SAS® System for the analysis of laboratory and field experiments commonly involve statistical inferences about treatment (fixed) effects using ANOVA, mean separation and contrasts, etc. Such a long trend has been seen in popular textbooks such as Steel et al. (1997) in which the use of PROC GLM for the analysis of examples is predominant. It is an established fact that PROC GLM is based on a strict fixed-effect model, but most field experimentations incorporate both fixed and random effects. Hence, the development of another SAS® procedure, PROC MIXED, which offers a more powerful and flexible of linear combinations of fixed and random effects which give an accurate validity of conclusions and recommendations.

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APPENDIX 1

A. SAS Studio Syntax and Output for PROC GLM (FIXED MODEL)

```

SAS® Studio
*Program 1
CODE LOG RESULTS
input irrig $ fruitwt1-fruitwt8;
array fw[8] fruitwt1-fruitwt8;
do bloc = 1 to 8;
    fruitwt = fw[bloc];
    logfw = log(fruitwt);
    output;
end;
drop fruitwt1-fruitwt8;
datalines;
11 Trickle 450 469 249 125 280 352 221 251
12 Spray 331 402 183 70 258 281 219 46
13 Basin 358 512 281 58 352 293 283 186
14 Flood 245 380 263 62 336 282 171 98
;
run;
/* PROC GLM analysis */
ods html;
ods graphics on;
proc glm data=methods;
    class bloc irrig;
    model fruitwt = bloc irrig;
    lsmeans irrig / pdiff=all cl; /* pairwise differences and confidence limits */
run;

```

Output window shows the data table:

Line	irrig	fruitwt1	fruitwt2	fruitwt3	fruitwt4	fruitwt5	fruitwt6	fruitwt7	fruitwt8
11	Trickle	450	469	249	125	280	352	221	251
12	Spray	331	402	183	70	258	281	219	46
13	Basin	358	512	281	58	352	293	283	186
14	Flood	245	380	263	62	336	282	171	98

Class Level Information		
Class	Levels	Values
bloc	8	1 2 3 4 5 6 7 8
irrig	4	Basin Flood Spray Trickle

Number of Observations Read	32
Number of Observations Used	32

The GLM Procedure

Dependent Variable: fruitwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	404191.0625	40419.1063	19.42	<.0001
Error	21	43709.1562	2081.3884		
Corrected Total	31	447900.2188			

R-Square	Coeff Var	Root MSE	fruitwt Mean
0.902413	17.49025	45.62224	260.8438

Source	DF	Type I SS	Mean Square	F Value	Pr > F
bloc	7	366377.9688	52339.7098	25.15	<.0001
irrig	3	37813.0938	12604.3646	6.06	0.0039

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

irrig	fruitwt LSMEAN	LSMEAN Number
Basin	290.375000	1
Flood	229.625000	2
Spray	223.750000	3
Trickle	299.625000	4

Least Squares Means for effect irrig Pr > t for H0: LSMean(i)=LSMean(j)				
Dependent Variable: fruitwt				
i/j	1	2	3	4
1		0.0644	0.0378	0.9769
2	0.0644		0.9938	0.0276
3	0.0378	0.9938		0.0157
4	0.9769	0.0276	0.0157	

irrig	fruitwt LSMEAN	95% Confidence Limits	
Basin	290.375000	256.831045	323.918955
Flood	229.625000	196.081045	263.168955
Spray	223.750000	190.206045	257.293955
Trickle	299.625000	266.081045	333.168955

B. SAS Studio Syntax and Output Using PROC MIXED (RANDOM MODEL)

SAS® Studio

*Program 1

CODE LOG RESULTS OUTPUT DATA

```

4      do bloc = 1 to 8;
5          fruitwt = fw[bloc];
6          logfwt = log(fruitwt); /* optional transformation */
7          output;
8      end;
9      drop fruitwt1-fruitwt8;
10     datalines;
11 Trickle 450 469 249 125 280 352 221 251
12 Spray   331 402 183 70  258 281 219 46
13 Basin   358 512 281 58  352 293 283 186
14 Flood   245 380 263 62  336 282 171 98
15 ;
16 run;
17 /* PROC MIXED with random blocks */
18 ods html;
19 ods graphics on;
20 proc mixed data=methods method=type3;
21     class bloc irrig;
22     model fruitwt = irrig;          /* fixed effect: irrigation */
23     random bloc;                  /* random effect: block */
24     lsmeans irrig / pdiff=all cl;  /* pairwise differences with confidence limits */
25 run;
26 ods graphics off;
27 ods html close;

```

The Mixed Procedure

Model Information	
Data Set	WORK.METHODS
Dependent Variable	fruitwt
Covariance Structure	Variance Components
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
bloc	8	1 2 3 4 5 6 7 8
irrig	4	Basin Flood Spray Trickle

Dimensions	
Covariance Parameters	2
Columns in X	5
Columns in Z	8
Subjects	1
Max Obs per Subject	32

Dimensions	
Covariance Parameters	2
Columns in X	5
Columns in Z	8
Subjects	1
Max Obs per Subject	32

Number of Observations	
Number of Observations Read	32
Number of Observations Used	32
Number of Observations Not Used	0

Type 3 Analysis of Variance								
Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
irrig	3	37813	12604	Var(Residual) + Q(irrig)	MS(Residual)	21	6.06	0.0039
bloc	7	366378	52340	Var(Residual) + 4 Var(bloc)	MS(Residual)	21	25.15	<.0001

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
irrig	3	21	6.06	0.0039

Least Squares Means									
Effect	irrig	Estimate	Standard Error	DF	t Value	Pr > t	Alpha	Lower	Upper
irrig	Basin	290.37	42.7872	21	6.79	<.0001	0.05	201.39	379.36
irrig	Flood	229.62	42.7872	21	5.37	<.0001	0.05	140.64	318.61
irrig	Spray	223.75	42.7872	21	5.23	<.0001	0.05	134.77	312.73
irrig	Trickle	299.62	42.7872	21	7.00	<.0001	0.05	210.64	388.61

Differences of Least Squares Means										
Effect	irrig	_irrig	Estimate	Standard Error	DF	t Value	Pr > t	Alpha	Lower	Upper
irrig	Basin	Flood	60.7500	22.8111	21	2.66	0.0145	0.05	13.3117	108.19
irrig	Basin	Spray	66.6250	22.8111	21	2.92	0.0082	0.05	19.1867	114.06
irrig	Basin	Trickle	-9.2500	22.8111	21	-0.41	0.6892	0.05	-56.6883	38.1883
irrig	Flood	Spray	5.8750	22.8111	21	0.26	0.7993	0.05	-41.5633	53.3133
irrig	Flood	Trickle	-70.0000	22.8111	21	-3.07	0.0058	0.05	-117.44	-22.5617
irrig	Spray	Trickle	-75.8750	22.8111	21	-3.33	0.0032	0.05	-123.31	-28.4367