Addis Ababa University
Office of Graduate Program

Faculty of Science
Department of Statistics

APPLICATIONS OF REPEATED MEASURES ANALYSIS ON *ENSET* PLANT
(Ensete ventricousum)

By
Addisu Fekadu

A THESIS SUBMITTED TO THE OFFICE OF GRADUATE PROGRAMMES OF ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE IN STATISTICS

July, 2008

DECLARATION

I, the undersigned, declare that the thesis is my original work, has not been submitted to any university anywhere for the award of any academic degrees and all sources of material used for the thesis have been duly acknowledged.

Name: Addisu Fekadu
Signature: 28/07/08
Place: Faculty of Science, Addis Ababa University
Date: July, 2008

This thesis has been submitted for examination with my approval as a University advisor.

....
Girma Taye, (PhD)

Addis Ababa University
Office of Graduate Program

Faculty of Science
Department of Statistics

APPLICATIONS OF REPEATED MEASURES ANALYSIS ON *ENSET* PLANT
(Ensete ventricousum)

By
Addisu Fekadu

Approved by the Board of Examiners:

Girma Taye, (PhD)
Advisor Signature

Prof. Eshetu Wencheko
Examiner **Signature**

MK Sharma
Examiner MKSh
Signature

APPLICATIONS OF REPEATED MEASURES ANALYSIS ON *ENSET* PLANT
(Ensete ventricousum)

BY

ADDISU FEKADU

DEPARTMENT OF STATISTICS

FACULTY OF SCIENCE

A THESIS SUBMITTED TO THE OFFICE OF GRADUATE PROGRAMMES OF ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE IN STATISTICS

ADDIS ABABA

July, 2008

Table of Contents

Page

Table of Contents *(Continued)*

List of Abbreviations

AIC – Akaike's Information Criteria

ANOVA – Analysis of Variance

AR (1) – Autoregressive Process of Order 1

ARCS – Areka Research Sub Center

BIC –Schwarz's Bayesian Information Criteria

CS – Compound Symmetry

CSA – Central Statistical Authority

DF – Degrees of Freedom

G-G – Greenhouse - Geisser

GLS – Generalized Least Squares

H-F – Huynh-Feldt

MANOVA – Multivariate Analysis of Variance

ML – Maximum Likelihood

MS – Mean of Squares

REML – Restricted/Residual Maximum Likelihood

SAS – Statistical Analysis System

SNNPR–South Nations, Nationalities and Peoples Region

SS – Sum of Squares

SS & CP – Sum of Squares and Cross Products

UNS – Unstructured

List of Tables

DEFINITION OF TERMS

Amicho - the fleshy inner portion of the *inset* corm which may be cooked and eaten separately, testing similar to potato.

Bulla – the small amount of water insoluble starchy product that may be separated from *kocho* during processing by squeezing and decanting the liquid. It is eaten as porridge.

Clone or variety – a distinct type or grouping of plants within a species separable from other types by some form of heritable trait, be it visual, chemical or other. New plants of clones are usually reproduced asexually, i.e., without utilizing flowering and seed production.

Corm - an enlarged fleshy structure at the base of the plant which is used to make *Amicho*, which can be added to the *kocho* or from which new shoots emerge following destruction of the dominant shoot.

Kocho - the pulp of the *enset* psusostem derived by scraping the individual pieces and excluding the fiberous remains. *Bulla* may or may not be extracted and the *Amicho* may or may not be included. The raw mash is chopped and fermented. The flat-bread made from the stem is called *Kocho*.

ABSTRACT

Repeated measures analyses have become the most interesting areas in psychological, health and agricultural researches. Repeated measures data are measurements taken several times from the same subject. Such data tend to be serially correlated. Measurements taken close in time are potentially highly correlated than those taken far apart in time. Hence, they require special methods of analysis. In this paper, four major approaches are used to analyze repeated measurements taken from thirty three varieties of *enset* plants each measured at four successive time points. The study provides summary statistics, results based on repeated measures analysis of variance (Split-plot in time ANOVA), multivariate analysis of variance (MANOVA), and mixed model methods. Each method is described briefly. In order to apply the repeated measures ANOVA, compound symmetry assumptions of covariance structures should be met. Whether the data fulfils this structure is tested. For those data which do not satisfy this criterion, the degree of freedom is adjusted for F test statistics by Huynh-Feldt (H-F) or Greenhouse-Geisser (G-G) epsilons. The multivariate approach is less restrictive but lacks power given that the repeated measures ANOVA assumptions are satisfied and the sample size is small. In all the methods considered, SAS was used to analyze the data. The results using univariate, multivariate and mixed approaches of repeated measurements of *enset* plants show that the main effects of variety and time as well as the interaction effect of variety by time were found to be significant. It was found that mixed model approach provides a very flexible environment in which the covariance structure can be modeled. Besides, the mixed model permits selection of the covariance structure that best fits the data at hand and enables to compute efficient estimates of fixed effects and valid standard errors of the estimates.

ACKNOWLEDGEMENT

I wish to express my sincere and heartfelt gratitude to my advisor, Dr. Girma Taye, for suggesting the topic of this thesis, for his valuable advice and unlimited assistance throughout my study.

My thanks are also due to Dr. Mulugeta Diro and staff of Areka Agricultural Research Sub Center, Ato Zerihun Yemataw and Ato Mikias Yeshitila who contributed in providing and organizing the data the way I wanted and briefing me about the data during my visit.

I would like to thank my employer, Nib Insurance Company (S.Co) for granting me sponsorship for the M.Sc. program.

Finally, I would like to extend my thanks to members of the Department of Statistics of Addis Ababa University, my family, and all my friends and colleagues for their special assistance and encouragement.

CHAPTER ONE

INTRODUCTION

1.1 About *Enset* Plant

Enset (Ensete ventricousum (welw.)) is a herbaceous perennial plant. Ensete is the name of the genus (Cheesman, 1947) whereas *enset* is the vernacular used in the Amharic Language. Ensete belongs to the family Musaceae under which Musa, the genus of banana, belongs. *Enset* resembles banana for both have underground stem (corm), a concentric bundle of leaf sheaths (pseudostem) and large leaves.

The lowland and mountain areas of Uganda, Tanzania, the Sudan (Smeds, 1955) and Asia (Purseglove, 1972) are amongst areas suggested as the centers of origin for Ensete. However, Vavilov (1957) and Kuls (cited by Westphal, 1975) reported Ethiopia as the center of origin for Ensete, the country in the world where *enset* cultivation as food crop is recorded.

According to Taye et al. (1967), *enset* is best adapted to altitudes between 1500 and 3000m. It was also reported that *enset* performed well under irrigation at an altitude of 1200m. Its cultivation is concentrated in southern and southwestern parts of the country where it plays a significant economic and social role.

About 15 million people of the Ethiopian population depend on *enset* as their source of staple or co-staple food in the form of *kocho, bulla* and *amicho* (Spring, 1996). Over 180 thousand hectars were estimated to be allocated to *enset* planting by private holders in the country in 1990/91 crop season (Central Statistical Authority (CSA), 1995).

Although its food value is low in protein content (Agren and Gibsen, 1968), *enset* appears to be a productive crop (progress report, ARC, 1995, unpubl.). *Enset* is a good source of income, a measure of wealth, a cultural medicine and feed for livestock. Moreover, it has environmental value as its system contributes to sustainable agriculture. The fiber, by-product when *enset* is processed has also an economic value.

Enset is relatively tolerant to draught. It can be harvested at any time of the year and the processed product can be stored for a long time without spoilage.

Because of these characteristics it is called a life saving crop. Thus, yield is sustainable and *enset* undoubtedly can serve as a crop for food security against famine. This could be one of the reasons for *enset* expansion to non-traditional areas nowadays. Even though, *enset* is of significant importance to the Ethiopian economy, very little attention has been given to its improvement. Hence, more studies and researches should be done on this plant to improve its yields.

In this thesis four statistical methods are used to analyze repeated measurements of *enset* plant. Summary statistics deal mainly with descriptive aspects of the data. In univariate analysis of variance (ANOVA), the underlying assumptions will be checked first and hypothesis tests of main and interaction effects will follow. Next, the multivariate analysis of variance (MANOVA) methods are used to test the within-subject effects. Finally, the mixed model is used to test the main and interaction effects after modeling the covariance structure of the data.

1.2 Statement of the Problem

There are several studies conducted on *enset* plant. However, most of them focused on biological aspects and simple descriptive analyses only. The breeders and agronomists do not usually use well-founded and appropriate statistical methods to justify whether the different varieties of *enset* give rise to significant differences in yield and whether variety and time have significant interactions. Usually they collect repeated measurements from the various *enset* clones over time but they often judge the difference among varieties based on observations, intuitive approach and descriptive statistics for the purpose of evaluation, breeding and maintenance of varieties. The author of this work has not come across crop researches that use the classical methods of repeated measures analysis in Ethiopia. Therefore, this study demonstrates how the right statistical methodology can be applied in crop research, in this case a research on *enset*.

1.3 Objectives of the Study

Using the *enset* pseudostem circumference measurements collected from various varieties repeatedly over four successive time points, this paper attempts to illustrate appropriate and sophisticated statistical methods to analyze repeated measurements. The specific objectives are to:

- ✓ show how the variety means are changing over time using the summary statistics (descriptive methods and graphical display of the different variety means on the same graph);
- ✓ test the main effects and interaction effects of variety and time using Univariate, Multivariate and Mixed Model Approaches of repeated measures analysis on the *enset* data;
- ✓ compare the various approaches and select the one which would provide better results.

CHAPTER TWO
LITERATURE REVIEW

2.1 Concepts of Summary Statistics

Summary statistics are single values that summarize some aspects of the subject's response profile. The summary measure to be used needs to be chosen before the analysis of the data and should, of course be relevant to the particular questions of interest in the study. In clinical trial work, for example, Frison and Pockock (1992) argued that the average response to treatment over time is often likely to be the most relevant summary of the repeated measurements.

Another approach of summary statistics is to plot means of each subject under different treatment conditions. A method often employed, particularly in medical publications, is to plot means by treatment group for every time point. When the number of individual curves is large, it may be more helpful to use a small number of representative curves. Jones and Rice (1992) suggested to use principal components analysis to select a small number of the growth curves which can explain most of the variability. Specially, those curves corresponding to the units with the minimum, maximum and median principal component scores on the first one or two principal components will generally give an adequate summary of all the curves.

2.2 General Background on the Mixed Model Analysis of Variance (ANOVA)

There are limitations to the utility of the mixed model ANOVA when the assumptions about the model are violated (e.g. Davidson, 1972; Morrison, 1976; Winer, 1971). One of the more lucid reviews of the problem was published by McCall and Appelbaum (1973). They outlined the fundamental problems in the use of traditional mixed model ANOVA for repeated-measures designs, and reviewed the available alternatives. Their review emphasized the utility of multivariate ANOVA (MANOVA) for statistical hypothesis tests that are valid even when the mixed model assumptions are violated.

According to Hertzog and Rovine (1985), a decision regarding whether to use mixed model ANOVA or MANOVA significance tests represents just one aspect of a more complex process. In addition to whether mixed model assumptions are violated, the following additional questions need to be considered as well: (1) Are there multiple dependent measures to be analyzed, so that a true multivariate repeated-measures analysis, not just a multivariate significance test for univariate data, is required? (2) Is it in general necessary or desirable to use omnibus hypothesis testing (i.e., testing a null hypothesis that all effects associated with a given factor [independent variable] are null), given the nature of the substantive hypotheses to be tested? (3) If omnibus testing is employed, what are the appropriate procedures for post-hoc analysis of significant effects?

The assumption of homogeneous variance and covariance rarely holds in many common applications of repeated measures designs. In longitudinal data, for example, covariances among the variables often decrease with increasing separation of measurement occasions. Validation of the assumption introduces a positive bias into the F-test. One is more likely to commit a Type I error than is indicated by the nominal Type I error rate (McCall and Appelbaum, 1973). With several within-subject levels and severe violation of the assumption, the magnitude of the positive bias introduced can be quite substantial (Box, 1954).

2.3 General Background on Multivariate Analysis of Variance (MANOVA)

For designs with two or more within-subject factors, their interactions are handled by the generation of orthogonal interaction contrasts (e.g. multiplying the main effect contrasts for the two factors to create interaction contrasts). Using these contrast weights new linear combinations for interaction are created, and then calculating MANOVA significance tests in exactly the same manner as for the within-subjects main effects (McCall and Appelbaum, 1973).

McCall and Appelbaum (1973), among others, emphasized the utility of MANOVA significance tests in repeated measures ANOVA. Their argument may be summarized as follows:

(a) the mixed model assumptions are rarely satisfied, hence mixed model ANOVA is not justifiable as a general data analytic technique;

(b) adjustments for the degrees of freedom of mixed model omnibus test (as promoted by Greenhouse and Geisser, 1959) are unnecessarily conservative; and therefore

(c) the MANOVA approach could be recommended as a general analysis strategy, since it makes less restrictive assumptions (with acceptable behavior of the significance tests if the assumptions are violated) and yet provided acceptable statistical power.

The MANOVA test provides more valid Type I error rates and better statistical power given severe violations of the circularity assumption (e.g., Rogan et al., 1979; Scheifly and Schmidt, 1978).

2.4 Test for Serial Correlation in Univariate Repeated Measures Analysis

Topics related to the analysis of repeated measures designs have received a great deal of attention in recent literature. Here, the works related to the effect of the invalidity of the variance assumptions on the repeated measures F-test is presented and the effect of a certain invalid structure on the analysis is emphasized (Hearne, et al., 1983).

Confusion has existed regarding the implications these assumptions have for the original covariance matrices. Huynh and Mandeville (1979) pointed out that the assumptions do not require equality of the original matrices, but they do require same variance for $X_i - X_j$ across groups, i.e., the same 'Type H' structure (Huynh and Feldt, 1970). The situation of equal matrices across groups and compound symmetry of the common matrix is a special case satisfying the assumptions. Huynh and Feldt suggest that the form of the covariance matrices should always be examined when data from repeated measures designs are analyzed. Departures from the 'type H' assumption can produce significantly inflated probability levels as demonstrated by the serial correlation pattern. The serial correlation pattern has a wide range of applicability, especially in situations where observations are recorded over time. The likelihood ratio test could be used to identify the covariance structure. However, the serial correlation pattern is not the only structure that might be considered. More complicated autoregressive models might be more plausible in certain situations. It might be possible to extend these results to more general designs (Hearne, et al., 1983).

2.5 Repeated Measures Analysis in Animal Science

Gorgulu and Sahiler (2001) discussed the advantages of using repeated measures analysis on animal experiment. Few animals may be available (or few used because of complex technique applied on animals while taking measurements) in experiments with non-random repeated measurement (e.g. *p* animals in each of *r* treatment groups, each measured in *p* periods). In such cases, the use of summary statistics for each animal to eliminate the time factor, or ordinary univariate split-plot tests of the treatment means or multivariate analysis is not advisable, because comparison of the treatments are not sufficiently sensitive to any of those procedures. The problem is that main effects of treatments must be tested by the mean square for the animals within treatments, which is inflated by positive correlations among repeated observations. Even conditional tests (e.g. comparisons of treatments within periods), as well as tests of means of summary statistics cannot be very sensitive, because, with low replication, the standard errors mean differences are not much smaller than the ordinary (error) standard deviation among animals treated alike, without the influence of correlations induced by repeated measurement. Too small number of animals leaves few degrees of freedom for error, either reducing statistical power drastically or preventing multivariate analysis entirely. In such cases, the primary benefit of a repeated measures design is statistical power relative to sample size, which is important in many real researches. Repeated measures designs use the same subjects throughout different treatments and thus, require fewer subjects overall. Because the subjects are the same, the variance due to subjects is partitioned into the error variance term, thereby making any statistical test more powerful.

2.6 E*nset* Crop Assessment

Shank and Ertiro (1996) reported that data from Wolaita Agricultural Development Unit indicated a positive relationship between measurements plant pseudostem girth and height with plant *kocho* yield. Choosing a 90% confidence interval for the standard error of an estimate, it was statistically predicted that 67 plants, sampled over a range of sizes, would be sufficient to construct the model. Sixty-five samples were taken at random throughout the *enset* growing areas by asking the household whether they had *enset* under fermentation from a single plant. The total *enset* products were weighed and sampled for moisture content and the size of the plant from which it came was measured. Individual plant yields varied 114.7 kg (298cm circumference × 303 cm high plant) down to 3 kg (58cm × 93 cm plant). Correlation of yield with circumference was 87% and with the pseudostem height 83%. Both the analysis of variance for linear regression and the coefficients for circumference and height were highly significant in predicting plant yield. The derived model

$$\text{PLANT YIELD (kg)} = -36.5 + 0.23 \times \text{CIRCUMFERENCE (cm)} + 0.19 \times \text{HEIGHT (cm)}$$

was sufficient to account for 82% of the observed variation in plant yield. Hence, from the above results it can be concluded that the pseudostem circumference and the pseudostem height can be used to determine the yield of *enset* plant since both have strong correlation with the yield.

The literature reviewed so far are some of the conditions under which repeated measures analyses are applied and their applications in various disciplines. Furthermore, the derived model of the linear relationship between pseudostem circumference and height with *enset* yield was reviewed. The primary purposes of experiments in which the same subject is observed under each of the treatments is to provide a control on differences between subjects. Each subject serves as its own control. Responses of individual subjects to the treatments are measured in terms of deviations about a point which measures the average performance of that individual subject. Hence variability due to differences in the average responsiveness of the subjects is eliminated from the experimental error (Winer, 1971). Therefore, using repeated measurements of the *enset* plant to compare varieties gives better results than using different plants. However, the author couldn't find literatures using repeated measures analysis on crops locally. Hence, this thesis is concerned with applications of repeated measures analysis on the perennial plant *enset*. Thus, we follow methods that will be discussed in subsequent chapters.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Data

The data used in this paper are collected at *Areka* Research Sub-Center (ARSC). The Sub-Center is found in Southern Nations, Nationalities and Peoples' Regional Government in North *Omo* Zone. *Areka* is the capital of *Boloso Sorie Woreda* which is 30 km away from the town of *Wolaita Soddo*, on the way to *Hosaina*.

A plot of land is prepared so as to make it as homogeneous as possible. The four replicates of each of the thirty three *enset* varieties are transplanted to the plot in a randomized fashion using completely randomized design. The plants are grown on the plot for one year. The repeated measurements of pseudostem circumference are then taken four times every six months from each *enset* plant.

Data Layout

Data layout for two-factor repeated measurements design

		Time points					
Variety	Plant	1	2	.	.	.	t
1	1	Y_{111}	Y_{112}	.	.	.	Y_{11t}
1	2	Y_{121}	Y_{122}	.	.	.	Y_{12t}
.	.	.	.				.
.	.	.	.				.
.	.	.	.				.
1	n_1	Y_{1n_11}	Y_{1n_12}	.	.	.	Y_{1n_1t}
.	.	.	.				.
.	.	.	.				.
.	.	.	.				.
s	1	Y_{s11}	Y_{s12}	.	.	.	Y_{s1t}
s	2	Y_{s21}	Y_{s22}	.	.	.	Y_{s2t}
.	.	.	.				.
.	.	.	.				.
.	.	.	.				.
s	n_s	Y_{sn_s1}	Y_{sn_s2}	.	.	.	Y_{sn_st}

where $i = 1, 2, \ldots, s$ variety; $j = 1, 2, \ldots, n_i$ plant in variety i

$k = 1, 2, \ldots, t$ time points (with $n = \sum_{i=1}^{s} n_i$)

Y_{ijk} is the pseudostem circumference of the i^{th} variety of plant j at time k.

3.2 Limitations of the Study

- The repeated measurements taken from each clone are not taken at exactly the same time. i.e., since it takes certain time to measure the pseudostem circumference of each *enset* plant and there is limited number of manpower who takes the measurements, the time point at which one clone is measured varies from the other which makes difficult the absolute comparisons among the different *enset* clones;
- The age of the *enset* clones which were transplanted from different locations may also vary.

3.3 Methodology

When several measurements are taken on the same experimental unit (person, plant, machine, and so on), the measurements tend to be correlated with each other. When the measurements represent qualitatively different indicators such as weight, length, and width, this correlation is best taken into account by use of multivariate methods, such as multivariate analysis of variance. When the measurements can be thought of as responses to levels of an experimental factor of interest, such as time, treatment, or dose, the correlation can be taken into account by performing a repeated measures analysis of variance.

Repeated measures data arise when time sequences of observations of the same dependent variable are made on each of a number of experimental units (usually subjects) possibly allocated to one of several treatments. The investigator may also vary systematically the conditions under which the repeated measurements are made, thus introducing one or more within-subject factors into the design.

Repeated measures analysis is a type of analysis of variance in which variation between experimental units (often called "between-subjects" variation) and variation within units (called "within-subjects" variation) are examined. Between-units variation can be attributed to the factors that differ across the study groups (e.g., treatment, varieties, species and stock type). Within-units variation is any change, such as an increase in circumference, height, etc. that is observed in an individual experimental unit.

The methods used to analyze repeated measures data range from simple to complex with a particular approach often being specific to a particular discipline.

There are a number of approaches that could be considered in order to analyze repeated measures data. The major ones are:

1. Summary statistics;
2. Univariate Analysis of Variance (Split-plot-in-time ANOVA);
3. Multivariate Analysis of Variance(MANOVA); and
4. Mixed Model Approach.

3.3.1. Summary Statistics

A more relevant, but still relatively straightforward approach to the analysis of repeated measures data is that involving the use of summary measures, sometimes known as response feature analysis. Here, the responses for each plant are used to construct a single value that summarizes some aspect of the plant's response profile (in some cases more than a single summary measure may be used).

Another useful initial step in the analysis of repeated measures data is to graph the data. In most repeated measures analyses, the means are plotted against time for each variety, by differentiating between varieties using different colors or labeling the varieties.

By plotting the treatment mean profile against time, we can also see the treatment by time interaction effects. We plot the treatment means against time for each subject. In this way, we can examine the form that the interactions take.

3.3.2 Univariate Analysis of Variance (Split-plot in time ANOVA) Method for Analyzing Repeated Measures

The univariate ANOVA for analyzing repeated measures involves modeling the data using the linear model.

$$Y_{ijk} = \mu + \alpha_i + b_{ij} + \tau_k + (\alpha\tau)_{ik} + e_{ijk} \tag{3.1}$$

Using this linear model, we are going to assume that the data for variety *i*, plant *j* at time *k* is equal to an overall mean μ plus the variety effect α_i, the effect of the plant in the variety b_{ij}, the effect of time τ_k, the effect of the interaction between time and variety $(\alpha\tau)_{ik}$, and the error e_{ijk}.

Here:

$i = 1, \ldots, s$ is an index for variety membership

$j = 1, \ldots, n_i$ is an index for plant *j* within variety *i*

$k = 1, \ldots, t$ is an index for levels of time

Y_{ijk} is the response measurement at time *k* for plant *j* within variety *i*,

μ is overall mean,

α_i is the added fixed effect for variety *i*,

τ_k is the added fixed effect for time *k*,

b_{ij} is the random effect due to plant j within variety *i*, assumed to be $i.i.d \sim N\left(0, \sigma_b^2\right)$,

$(\alpha\tau)_{ik}$ is the added effect for the variety *i* by time *k* interaction and e_{ijk} is the random effect on time *k* for plant *j* within variety *i*.

Also b_{ij} and e_{ijk} are assumed to be independent of one another. In repeated measures analysis, the b_{ij} are often called the between-subject effects and e_{ijk} are called the within-subject effects.

The errors e_{ijk} are independently sampled from a normal distribution with mean 0 and variance σ^2.

The effect of time does not depend on the plant; that is, there is no time by plant interaction. We need this assumption so that the results will not depend on which plant we are looking at.

Univariate analysis of variance (ANOVA) is the method most commonly applied to repeated measures data that makes comparisons of between and within-subject means. It treats the data as if they were a split-plot design with the between-subject variability as main plot and time as sub-plot, hence the name split-plot in time analysis. If measurements have equal variance at all times and if pairs of measurements on the same subject are equally correlated, regardless of the time lag between the measurements, the univariate ANOVA is valid from a statistical point of view, and, in fact, yields an optimal method of analysis. However, measurements close in time are often more highly correlated than measurements far apart in time, which will invalidate tests for effects involving time. The inherent dependence that is associated with repeated measures data introduces extra complications into the analysis. Unfortunately, the simplifying properties arising from data which are independently and identically distributed can no longer be relied up on. To yield conclusions which are valid, the analyst must take into account the possible dependence within subjects.

Another problem of applying standard ANOVA analysis method in repeated measures data is that it would regard the groups as a factor on different levels, and more importantly, it would regard time as a factor on *t* levels. One of the difficulties with this approach is that the allocation of times to the *t* observations within each subject cannot be randomized. But randomization of the various levels of a factor is an essential requirement of ANOVA. Usually ANOVA assumes that group factors are randomized within blocks. However, the time points which we regarded as factor levels cannot be randomized, an essential requirement of ANOVA. They must follow their natural sequence. The first measurement is the first measurement, it cannot be taken third. In general, these extra complications mean that the simple univariate ANOVA F-tests will no longer be valid.

In this section we discuss the use of univariate analyses that takes into account the multivariate nature of many repeated measures data sets following the assumptions regarding the properties of these types of data.

3.3.2.1 Assumptions about Analysis of Variance in Repeated Measures Designs

The validity of the F-test rests upon a set of assumptions concerning the nature of the underlying sources of variation and covariation or, equivalently, variances and covariances. Those, in turn, follow in part from assumptions made about the model, that is the underlying distribution of the observations.

3.3.2.2 Matrices to Summarize Assumptions about Variance – Covariances

The theoretical variance-covariance within-subject matrix $\sum_x$ is defined as

$$\underset{t\times t}{\sum}{}_x = \begin{pmatrix} \sigma_{11} & \sigma_{12} & . & . & . & \sigma_{1t} \\ \sigma_{21} & \sigma_{22} & . & . & . & \sigma_{2t} \\ . & & & . & & . \\ . & & & & . & . \\ . & & & & . & . \\ \sigma_{t1} & \sigma_{t2} & . & . & . & \sigma_{tt} \end{pmatrix}$$

The entries on the main diagonal are population variances, which are sometimes written as $\sigma_1^2, \sigma_2^2, \ldots \sigma_t^2$.

The sample variance - covariance matrix for a design with t treatment conditions is

$$\underset{X}{\hat{\sum}} = S_X = \begin{pmatrix} S_{11} & S_{12} & . & . & . & S_{1t} \\ S_{21} & S_{22} & . & . & . & S_{2t} \\ . & & & . & & . \\ . & & & & . & . \\ . & & & & . & . \\ S_{t1} & S_{t2} & . & . & . & S_{tt} \end{pmatrix}$$

The entries on the main diagonal are the within-treatment sample variances and may be written as

$S_1^2, S_2^2, \ldots, S_t^2$.

The entries off the main diagonal of S_x are the covariances and are sometimes written in the general form $s_{jj'}$

Certain patterned covariance matrices are of interest in order to make explicit the assumptions underlying repeated measures designs. In particular, the analysis of variance makes certain assumptions about the variances and covariances in a repeated measures design and about the relationships among those values. The most general matrix which best represents the case when all of those assumptions are met is called a Huynh-Feldt (Type H) matrix. In general, a type H matrix will have the property that

$$\frac{\sigma_{jj} + \sigma_{j'j'} - 2\sigma_{jj'}}{2} = \lambda \text{ for } j \neq j' \tag{3.2}$$

This relationship defines what is called circularity. Thus, type H matrices define a general class of matrices which includes circular matrices. That is, circularity requires that the sum of any two group variances minus twice their covariances equals a constant (2λ) and that this is true for all pairs of groups on which there are repeated measures. The scalar λ depends upon the relationship between groups and the scale of measurement, as do the variances and covariances.

To illustrate, suppose that a set of t vectors of matrix $\mathbf{X}_{(n\times t)}$ have a covariance matrix $\sum_x$ which has a pattern generated by

$$\underset{t\times t}{\sum}{}_x = A + A' + \lambda I_t\,, \tag{3.3}$$

where

$$\underset{k\times t}{A} = \begin{pmatrix} a_1 & a_1 & . & . & . & a_1 \\ a_2 & a_2 & . & . & . & a_2 \\ . & . & . & & & . \\ . & . & & & . & . \\ . & . & & & . & . \\ a_t & a_t & . & . & . & a_t \end{pmatrix}$$

, where $a_1, a_2, \ldots, a_t$, and λ are any known arbitrary constants.

A matrix $\sum_x$ having the pattern defined in equation (3.3) will be called a type H matrix.

The circularity relationship also implies that

$$\sigma_{x_j - x_{j'}} = 2\lambda\text{, all } j \neq j'. \tag{3.4}$$

In words, circularity implies that the variance of the difference between any two vectors of **X** is a constant. Finally, for any type H matrix, the average of the variance $\bar{\sigma}_{jj}$ and the average covariance $\bar{\sigma}_{jj'}$ in the matrix differ by a constant,

$$\bar{\sigma}_{jj} - \bar{\sigma}_{jj'} = \lambda, \tag{3.5}$$

where $\bar{\sigma}_{jj} = \dfrac{\sum_{j=1}^{t} \sigma_{jj}}{t}$ and $\bar{\sigma}_{jj'} = \dfrac{\sum_{j<j'}^{t} \sigma_{jj'}}{t}$.

Since $\bar{\sigma}_{jj} - \bar{\sigma}_{jj'} = \lambda$ is the difference between an average variance and an average covariance, it expresses a residual error variance and since this difference is a constant, the requirement of homogeneity of residual error variances for all pairs of groups is met. Recall computing $MS_{residual}$ as a pooled residual error variance. That is, $MS_{residual} = \bar{S}^2 - \overline{Cov}$, where $\bar{S}^2$ is average of variances in sample covariance matrix and

$\overline{Cov}$ is average of covariances in sample covariance matrix.

A type H matrix which has the property of circularity, but in addition has the property that both the variances (σ_{jj}) and covariances ($\sigma_{jj'}$) are constant, is called *Compound Symmetry.* Compound symmetry is a special case of circularity. σ_{jj} and $\sigma_{jj'}$ are both constant but they need not be equal to each other. A matrix which has a compound symmetry will have circularity, but a circular matrix need not have compound symmetry; the condition of compound symmetry is more restrictive than circularity.

In order to check the assumptions of repeated measures designs, it is helpful to define the matrix property of sphericity from the property of circularity. This can be accomplished in terms of a normalized orthogonal transformation of the covariance matrix which converts $\sum_x$ to matrix $\sum_y$.

Let u and v be any two distinct vectors of matrix **M**. Then **M** is said to be orthogonal if $u v = 0$. A matrix is said to be orthonormal if its rows are both orthogonal and in normal form. That is, **M** is orthonormal when $u'u=1=v'v$ and $u'v=0$. One could construct an orthonormal matrix by using as rows the coefficients for orthogonal comparisons and then normalizing those coefficients. For example, with $t = 3$, the coefficients for two orthogonal comparisons would be

$$c_{11} = -1 \qquad c_{12} = 0 \qquad c_{13} = 1$$

$$c_{21} = 1 \qquad c_{22} = -2 \qquad c_{23} = 1.$$

Since $\sum c_{1j} = \sum c_{2j} = 0$ and $\sum c_{1j} c_{2j} = 0$, these two sets of coefficients can be used to define comparisons and the comparisons are orthogonal. They would be *orthonormal* if they were also normalized, i.e., of unit length. A vector is normalized by dividing each entry by the norm, or length, of the vector. If, for example, $c_1^{'}$ is the row vector

$$c_1^{'} = \begin{pmatrix} -1 & 0 & 1 \end{pmatrix},$$

the norm is $\left\| c_1^{'} \right\| = c_1^{'} c_1 = \left(\sum c_j^2 \right)^{1/2} = \left[(-1)^2 + (0)^2 + (1)^2 \right]^{1/2} = \sqrt{2}$

and the normalized vector is

$$c_1^{*'} = \frac{1}{\left\| c_1^{'} \right\|} c_1^{'} = \frac{1}{\sqrt{2}} \begin{pmatrix} -1 & 0 & 1 \end{pmatrix} = \begin{pmatrix} -\frac{1}{\sqrt{2}} & 0 & \frac{1}{\sqrt{2}} \end{pmatrix}$$

Note that the normalized vector has unit length. That is

$$\left\| c_1^{*'} \right\| = \left[\left(-\frac{1}{\sqrt{2}} \right)^2 + (0)^2 + \left(\frac{1}{\sqrt{2}} \right)^2 \right]^{1/2} = 1$$

The second vector,

$$c_2^{'} = \begin{pmatrix} 1 & -2 & 1 \end{pmatrix}$$

has length

$$\left\| c_2^{'} \right\| = \left(\sum c_j^2 \right)^{1/2} = \left[(1)^2 + (-2)^2 + (1)^2 \right]^{1/2} = \sqrt{6}$$

and in normalized form is

$$c_2^{*'} = \begin{pmatrix} \frac{1}{\sqrt{6}} & -\frac{2}{\sqrt{6}} & \frac{1}{\sqrt{6}} \end{pmatrix}$$

Thus, we may define an orthonormal coefficient matrix as

$$M^* = \begin{pmatrix} -\frac{1}{\sqrt{2}} & 0 & \frac{1}{\sqrt{2}} \\ \frac{1}{\sqrt{6}} & -\frac{2}{\sqrt{6}} & \frac{1}{\sqrt{6}} \end{pmatrix}$$

Each row in M^* provides the coefficients for a normalized comparison which in general is an expression of the form

$$c_1^* x_1 + c_2^* x_2 + c_3^* x_3,$$

where, $\sum c_j^* = 0$ and $\sum c_j^{*2} = 1.0$.

In M^*, then the rows are orthogonal and of unit lengths; the matrix $\mathbf{M}^*$ is orthonormal. For an orthonormal matrix,

$$\boldsymbol{M^* M^{*'} = I},$$

since $c_{j'}' c_j = 0$ (i.e., rows are orthogonal) and $c_j' c_j = 1$ (i.e., rows are normalized). An orthonormal matrix need not be square; in applications to problems associated with repeated measures designs, $\mathbf{M}^*$ will not typically be square. If $\mathbf{M}^*$ is orthonormal and $\sum_x$ is circular, then

$$M^* \sum_x M^{*'} = \lambda I \tag{3.6}$$

where

$$\lambda = \frac{1}{2}\left(\sigma_{jj} + \sigma_{j'j'} - 2\sigma_{jj'}\right)$$

and I is an identity matrix. A matrix having the form λI is said to be spherical. There is nothing complicated about a spherical matrix; it is simply a matrix with λ on the main diagonal and zero elsewhere. In obtaining $\sum_y = \lambda I$, one has the covariance matrix of a set of variables which are orthogonal (all covariances are zero) with constant variance. If $\sum_x$ is circular, $\sum_y = M^* \sum_x M^{*'} = \lambda I$ will have those simple properties.

3.3.2.3 Variance-Covariance Matrix Properties and Analysis of Variance Assumptions

Huynh and Mandeville (1979) have presented a succinct discussion of the assumptions regarding variance-covariance matrices with repeated measures designs. When summarized, the validity of the F-statistic is assured when the covariance matrix of the observations is circular in form. Equivalently, the covariance matrix of a variable which is a normalized orthogonal transformation of the observations is spherical in form when the assumptions are met. The matrix $M^* \sum_x M^{*'} = \lambda I$ is important because when $\sum_x$ is circular, $M^* \sum_x M^{*'} = \lambda I$ is spherical. The analysis of variance assumption of circularity of $\sum_x$ is tested by evaluating whether $M^* \sum_x M^{*'} = \lambda I$ is spherical. To make what is being presented specific to the analysis of variance, the matrix $M^* \sum_x M^{*'} = \lambda I$ will be considered as the covariance matrix $\sum_y$ of *(t-1)* vectors of matrix **Y** whose components are normalized orthogonal transformations of the original t vectors of data matrix **X**. That is, one may transform the analysis of variance of data matrix **X** into new matrix **Y** which is orthogonal. $\sum_y$ is the variance-covariance matrix of those *(t-1)* orthogonal vectors of **Y**.

When $\sum_x$ is circular, $\sum_y$ is spherical. That is, $\sum_y$ has a single variance on the main diagonal and zero for all covariances. It is not necessary to transform **X** to **Y** or $\sum_x$ to $\sum_y$ in order to evaluate the analysis of variance assumptions. The presentation here is for illustrative purposes only to help clarify the analysis of variance assumptions.

The analysis of variance hypothesis

$$H_o : \mu_1 = \mu_2 = \ldots = \mu_j = \ldots = \mu_t = \mu \tag{3.7}$$

can always be recast in terms of a set of *(t-1)* mutually orthogonal comparisons. For example, if $t = 3$, then two orthogonal comparisons can be used to evaluate the hypothesis

$$H_o^1 = \mu_1 - \mu_2 = 0$$

$$H_o^2 = \mu_1 + \mu_2 - 2\mu_3 = 0$$

The coefficients for these comparisons are

$$c_{11} = 1 \qquad c_{21} = -1 \qquad c_{31} = 0$$

$$c_{12} = 1 \qquad c_{22} = 1 \qquad c_{32} = -2$$

and **M*** as one possible coefficient matrix whose rows are a set of *(t-1)* mutually orthogonal normalized comparisons is, then,

$$M^* = \begin{pmatrix} \frac{1}{\sqrt{2}} & -\frac{1}{\sqrt{2}} & 0 \\ \frac{1}{\sqrt{6}} & \frac{1}{\sqrt{6}} & -\frac{2}{\sqrt{6}} \end{pmatrix},$$

since the norm of the first row vector (comparison 1) is $\sqrt{2}$ and for the second comparison it is $\sqrt{6}$.

In the general case, there are t vectors of **X**; under a normalized orthogonal transformation, the t vectors of **X** may be transformed into a set of *(t-1)*, vectors of **Y**,

$$\underset{n\times(t-1)}{Y} = \underset{n\times t}{X} \underset{t\times(t-1)}{M}^{*'} \tag{3.8}$$

where $\underset{t\times(t-1)}{M}^{*'}$ = a matrix whose rows are a set of normalized orthogonal comparisons, i.e,

M* is an orthonormal ($M^* M^{*'} = I_{t-1}$);

$\underset{n\times t}{X}$ = a matrix of basic observations;

$\underset{n\times(t-1)}{Y}$ = a matrix of transformed scores.

If $\mathbf{S_x}$ is the observed covariance matrix for **X**, then $\mathbf{S_y}$, the covariance matrix for **Y** is given by

$$\underset{(t-1)\times(t-1)}{S_y} = M^* S_x M^{*\prime} \tag{3.9}$$

Since the set of *(t-1)* normalized orthogonal comparisons are not unique, the **Y** matrix will not be unique. However, many of the important properties of **Y** will be invariant for all possible choices of **M***. In particular, any property of **Sy** which depends upon the characteristic roots will be invariant for all choices of $\mathbf{M}^*$. These include the trace and determinants of $\mathbf{S_y}$.

$\mathbf{S_x}$ estimates $\sum_x$ and $\mathbf{S_y}$ estimates $\sum_y$. It is important to remember that if $\sum_x$ is the type H matrix, $\sum_y$ will be spherical. To demonstrate this,

$$\sum_y = M^* \sum_x M^{*\prime} \tag{3.10}$$

And since $\sum_x$ is a type H matrix, one may rewrite

$$\sum_y = M^*(A + A' + \lambda I_t)M^{*\prime}$$

$$= M^* A M^{*\prime} + M^* A' M^{*\prime} + \lambda M^* M^{*\prime}$$

From the definition of the matrix A, it follows that

$M^* A' = [0]$ and $AM^{*\prime} = [0]$

since the sum of the entries in any row of **M*** is zero. Hence,

$$\sum_y = \lambda M^* M^{*\prime} = \lambda I_{t-1}. \tag{3.11}$$

Thus, the **X** matrix with a covariance matrix which is a type H matrix has been transformed into matrix **Y**, the covariance matrix of which is λI. That is, all of the **Y** matrix column vectors have a common variance λ and all of the covariances are zero. A matrix with these properties is spherical. Although a matrix **X** having a multivariate normal distribution may always be transformed to a set of independently distributed vectors, the resulting vectors need not have the same variance. This applies to the special case in which $\sum_x$ is circular that $\sum_y$ will be spherical.

From the point of view of the assumptions, $\mathbf{S_y}$ is of interest. $\mathbf{S_y}$ estimates $\sum_y$ and the assumption underlying the distribution theory of the F-ratio on the treatment effects is that

$$E(\mathbf{S_y}) = \sum_y = \lambda I_{t-1} \tag{3.12}$$

That is, $\sum_y$ is spherical and $\mathbf{S_y}$ provides an estimate of that spherical matrix. $\mathbf{S_y}$ can be obtained directly from the **Y** matrix. However, $\mathbf{S_y}$ is usually obtained as $M * S_x M*'$.

If $\mathbf{S_y}$ were exactly spherical, the variances would be equal and the covariance would be zero. Equivalently, the correlation between two vectors of matrix **Y** would be zero. However, $\mathbf{S_y}$ only estimates $\sum_y$ and can differ from a spherical matrix by chance even if $\sum_y$ is, in fact, spherical and $\sum_x$ is therefore, circular.

ANOVA table used in univariate Repeated Measures analysis is given below:

Table 3.1 ANOVA Table

Source	**d.f**	**SS**	**MS**	**F**
Variety	*s-1*	$SS_{variety}$	$\frac{SS_{variety}}{s-1}$	$\frac{MS_{variety}}{MS_{plant(within\ variety)}}$
Time	*t-1*	SS_{time}	$\frac{SS_{time}}{t-1}$	$\frac{MS_{time}}{MS_{error}}$
Plant (within Variety)	*n-s*	$SS_{plant\ (within\ variety)}$	$\frac{SS_{plant(within\ variety)}}{n-s}$	-
Variety × Time	*(s-1) (t-1)*	$SS_{Variety \times time}$	$\frac{SS_{Variety \times time}}{(s-1)(t-1)}$	$\frac{MS_{Variety \times time}}{MS_{error}}$
Error	*(n-s) (t-1)*	SS_{error}	$\frac{SS_{error}}{(n-s)(t-1)}$	-
Total	***nt -1***	$\mathbf{SS_{total}}$	-	-

$$SS_{total} = \sum_{i=1}^{s}\sum_{j=1}^{n_i}\sum_{k=1}^{t} y_{ijk}^2 - nt\bar{y}_{...}^{\ 2} \qquad SS_{Plant(within\ variety)} = n\sum_{i=1}^{s}\sum_{j=1}^{n_i} \bar{y}_{ij}^2 - t\sum_{i=1}^{s} n_i \bar{y}_{i.}^2$$

$$SS_{Variety\times time} = \sum_{i=1}^{s}\sum_{k=1}^{t} n_i \bar{y}_{i.k}^2 - nt\bar{y}_{...}^2 \qquad SS_{Variety} = \sum_{i=1}^{s} n_i \bar{y}_{i..}^2 - nt\bar{y}_{...}^2 \qquad SS_{time} = n\sum_{i=1}^{s}\sum_{j=1}^{n_i}\sum_{k=1}^{t} \bar{y}_{..k}^2 - nt\bar{y}_{...}^{\ 2}$$

where $n = \sum_{i=1}^{s} n_i$ = total number of plants,

s= number of varieties in the study and

t= number of times a single plant is measured.

The sources of variation include variety, plant (within variety) which is the effect of the plant within variety, the effect of time, the interaction between variety and time; and error which is individual error in the model. All these add up to a total.

3.3.2.4 Departure from Assumptions

A measure of the extent to which $\sum_x$ departs from circularity (and, therefore, $\sum_y$ departs from sphericity) has been proposed by Box (1954), and this is given by

$$\varepsilon = \frac{t^2(\bar{\sigma}_{jj} - \bar{\sigma}_{..})^2}{(t-1)\sum\sum(\bar{\sigma}_{jj'} - \bar{\sigma}_{j.} - \bar{\sigma}_{.j} + \bar{\sigma}_{..})^2} \tag{3.13}$$

The measure may also be expressed in terms of λ_i, the characteristic roots of the matrix $\sum_y$ as

$$\varepsilon = \frac{(\sum \lambda_i)^2}{(k-1)\sum \lambda_i^2}, \tag{3.14}$$

where

$\bar{\sigma}_{.j}$ = mean for column j

$\bar{\sigma}_{j.}$ = mean for row j

$\bar{\sigma}_{..}$ = mean for all entries

$\bar{\sigma}_{jj}$ = mean of main diagonal entries

When $\sum_x$ has maximum departure from circularity, $\sum_y$ will have maximum departure from sphericity. For $t=4$, for example, it becomes $\sum_y = \underset{(t-1)\times(t-1)}{C}$

where each element of C is a non-zero constant c.

$$\sum_y = \begin{bmatrix} c & c & c \\ c & c & c \\ c & c & c \end{bmatrix}$$

The characteristic roots of this equation are

$\lambda_1 = 3c, \quad \lambda_2 = 0, \quad \lambda_3 = 0$

For the general case ε will be given by the constant,

$$\varepsilon = \frac{9c^2}{(t-1)(9c^2)} = \frac{1}{t-1}$$

Thus, the range of the measure of departure from circularity is

$$\frac{1}{t-1} = \varepsilon \leq 1$$

In general, $\sum_x$ is unknown but may be estimated by $\mathbf{S_x}$, the sample covariance matrix of the t vectors of matrix **X**. In terms of the latter, one may estimate ε (Greenhouse-Geisser, 1959) as

$$\hat{\varepsilon} = \frac{t^2(\bar{S}_{jj} - S_{..})^2}{[(t-1)(\sum\sum S_{jj'}^2 - 2t\sum S_{j.}^2 + t^2\bar{S}_{..}^2)]}, \tag{3.15}$$

where

$\sum S_{jj'}^2$ = Sum of the squares of all entries of $\mathbf{S_x}$

$\bar{S}_{..} = (\sum\sum S_{jj'})/t^2$ = Grand mean of all entries

$\bar{S}_{j.} = \sum S_{jj'}/t$ = Row means

$\bar{S}_{jj} = \sum S_{jj}/t$ = Main diagonal mean

This estimate of ε tends to be biased for larger values of ε. Others (Huynh and Feldt, 1976; Huynh, 1978) recommend a less biased estimate of ε,

$$\tilde{\varepsilon} = \frac{n(t-1)\hat{\varepsilon} - 2}{(t-1)[n-1-(t-1)\hat{\varepsilon}]} \quad . \tag{3.16}$$

This latter measure, $\tilde{\varepsilon}$ may exceed 1. When this occurs, set $\tilde{\varepsilon} = 1$. It will be found that $\tilde{\varepsilon} \geq \hat{\varepsilon}$; $\tilde{\varepsilon}$ will always be equal to $\hat{\varepsilon}$ when $\hat{\varepsilon} = \frac{1}{t-1}$.

Box (1954) has shown that the overall F-statistic in the repeated measures designs is distributed as

$F[(t-1)\varepsilon, (n-1)(t-1)\varepsilon]$

In terms of $\hat{\varepsilon}$, F is approximately distributed as

$F[(t-1)\hat{\varepsilon}, (n-1)(t-1)\hat{\varepsilon}]$.

That is, by adjusting the degrees of freedom to take into account the degree to which the circularity assumption has been violated, an F-distribution can be approximated with any arbitrary covariance matrix. If one assumes that $\varepsilon = 1$, then the degrees of freedom for the F-distribution are *(t-1)* and *(n-1)(t-1)*.That is, the assumption of circularity is met and no adjustment is required. If the assumption has not been met, this gives the F-test a positive bias and the real level of significance will exceed the nominal level established by the experimenter. If one sets ε at its minimum value, the assumption is that $\sum_x$ deviates maximally from circularity and $\varepsilon = \frac{1}{t-1}$. At this extreme, one uses F [1, (n-1)] since $(t-1)\varepsilon = (t-1).\frac{1}{t-1} = 1$ it follows that $(n-1)(t-1)\varepsilon = (n-1)(t-1).\frac{1}{t-1} = (n-1)$.

In most instances, this adjustment is too extreme and the F-test will have a negative bias. However, it is reasonable to use this procedure, and if H_o is rejected no further steps are required since this test would have minimum statistical power and any other value for ε would lead to rejection of the null hypothesis. The use of F (1, n-1) has been labeled the Geisser – Greenhouse conservative test (Geisser-Greenhouse, 1959; Krik, 1982).

If one uses the unadjusted F and does not reject the null hypothesis, no further steps are necessary since that F-test has maximum power and the use of other values for ε would lead to non-rejection of H_o.

Uncertainty arises when the unadjusted F leads to rejection of the null hypothesis, and the maximum correction leads to non-rejection. It is under those circumstances that ε should be estimated and the adjusted degrees of freedom should be used.

3.3.2.5 Test of Assumptions

Mauchley (1940) introduced a procedure for testing the hypothesis that

$$M^* \sum_x M^{*'} = \sum_y = \lambda I$$

The test statistic is

$$W = \frac{|M^* S_x M^{*'}|}{[tr(M^* S_x M^{*'})/t-1]^{t-1}} = \frac{|S_y|}{[tr(S_y)/t-1]^{t-1}} \qquad (3.17)$$

where $|M^* S_x M^{*'}| = |S_y|$ is the determinant of $\mathbf{S}_y$, and

$tr(M^* S_x M^{*'}) = tr(|S_y|)$ is the trace of $\mathbf{S}_y$.

The range of W is $0 \le W \le 1$, the lower limit occurring when there is maximum departure from sphericity and the upper limit $W = 1$ corresponding to $\mathbf{S}_y$ being spherical. The smaller the numerical value of W, the greater is the departure of $\mathbf{S}_y$ from sphericity. Tables of the exact sampling distribution of the statistic W (under the hypothesis that $\sum_y$ is spherical) have been prepared by Nargarsenker and Pillai (1973).

A good approximation of the exact distribution of W may be obtained by the maximum-likelihood statistic

$$L = -d(n-1)(\ln W), \tag{3.18}$$

where

$$d = \frac{(2p^2 + p + 2)}{6p(n-1)} \quad , \quad p = t - 1 \tag{3.19}$$

The statistic L is approximately distributed as chi-square with degrees of freedom equal to

$\left[\left(\frac{p(p+1)}{2}\right)\right] - 1.$

The Mauchley test may be used to test the hypothesis that $\sum_x$ is circular by testing for sphericity in $\sum_y$.

From tables of the exact distribution of W one can find $W_{(1-\alpha)}(t-1, n)$.

At α level of significance, the decision is to reject the hypothesis of circularity, if $W_{cal} < W_{(1-\alpha)}(t-1, n)$.

To use the Chi-square approximation to the sampling distribution of W, we can transform W to L as follows:

$$d = 1 - \frac{2p^2 + p + 2}{6(p-1)} \text{ and } f = \frac{p(p+1)}{2} - 1$$

Hence, L can be calculated as in equation (3.18) above.

In this case, for a test having α, the decision rule is to reject the hypothesis of sphericity if

$L_{cal} > \chi^2_{(1-\alpha)}(f)$

3.3.2.6 Hypothesis Testing

1. The interaction between variety and time, or:

$H_0^1: (\alpha\tau)_{ik} = 0$ against H_1^1 : Not H_0^1 at α level of significance.

For $i=1, 2, \ldots, s$;

$k=1, 2, \ldots, t$.

We reject H_0^1 if $F_{cal}^1 = \dfrac{MS_{\text{variety}\times time}}{MS_{error}} > F_\alpha[(s-1)(t-1),(n-s)(t-1)]$

2. Let us suppose we had not found a significant interaction. Consider testing the null hypothesis that there are no variety effects, or

$H_0^2: \alpha_1 = \alpha_2 = \ldots = \alpha_s = 0$ against H_1^2 : Not H_0^2 at α level of significance.

We reject H_0^2 if $F_{cal}^2 = \dfrac{MS_{\text{variety}}}{MS_{plant(\text{within variety})}} > F_\alpha[(s-1),(n-s)]$.

3. Consider testing the effect effects of time, or to test

$H_0^3: \tau_1 = \tau_2 = \ldots = \tau_s = 0$ against H_1^3 : Not H_0^3 at α level of significance.

We reject H_0^3 if

$$F_{cal}^3 = \frac{MS_{time}}{MS_{error}} > F_\alpha[(t-1),(n-s)(t-1)].$$

3.3.3 Multivariate Analysis of Variance (MANOVA) Approach to Repeated Measures Analysis Based on Contrasts

3.3.3.1 General Multivariate Problem

Multivariate model can be written as

$$Y_i' = a_i' M + \varepsilon_i',\ i=1, \ldots, m, \quad \varepsilon_i' \sim N(0, \Sigma), \ldots\ldots (3.20)$$

Where Y_i' is the data vector of observations at the n time points from the ith unit,

a_i' is $(1 \times q)$ indicator vector of group membership,

M $(q \times n)$ matrix whose rows are the transposes of the mean vectors for each group

ε_i' is the error vector.

Multivariate analysis of variance (MANOVA) is an alternative to repeated measures ANOVA in which responses to the levels of the within subject variables are regarded as separate variables. Here, the repeated measures MANOVA approach regards the observations at different time points as separate variables.

In order to appreciate the perspective behind the multivariate approach, we consider a general case of a multivariate problem. Consider the following situation; we use the notation with two subscripts for convenience.

- Units are randomized into q groups.
- Data vector Y_{hl} is observed for the h^{th} unit in the l^{th} group
- Y_{hl} is assumed to satisfy $Y_{hl} \sim N(\mu_l, \Sigma)$,

  where μ_l is the mean response vector for group l and Σ is an arbitrary covariance matrix assumed to be the same for each group.
- There are r_l units in each group, so for group l, $h=1, \ldots, r_l$
- The components of Y_{hl} may not necessarily all be measurement of the same response. Instead, each component of Y_{hl} may represent the measurement of a different response. For example, suppose the units are birds of two species. Measurements on n different features of the birds may be taken and collected into a vector Y_{hl}, e.g., Y_{hl1} may be tail length, Y_{hl2} may be wing span, Y_{hl3} may be body weight, and so on. That is, the elements Y_{hlj}, $j=1, 2, \ldots, n$ may consist of measurements of different characteristics.

Of course, the longitudinal data situation is a special case of this set-up where the Y_{hlj} happen to be measurements of the same response (over time).

Clearly, the main interest is focused on comparing the groups on the basis of the responses that make up a data vector. Here, we compare all the different responses "simultaneously". In doing this, it would naturally be important to take into account that observations on the same unit are correlated.

In our statistical model, μ_l is the mean for data vectors (composed of the n different responses) observed on units in the l^{th} group. Thus, we may formally state our desire to compare the *n* responses 'simultaneously' as the desire to compare the *q* mean vectors μ_l, $l=1,\ldots, q$, on the basis of all their components. That is, we are interested in testing the null hypothesis

$H_o : \mu_1 = \ldots = \mu_q$ versus the alternative $H_1 : \mu_i \neq \mu_j$ for at least one pair $i \neq j$ (3.21)

As long as the *n* responses that make up a data vector are different and hence not comparable (e.g. cannot be "averaged"), this is the best we can do to address our general question.

3.3.3.2 Hotelling's T^2

The standard methods to test the null hypothesis (3.21) are simply generalizations of standard methods in the case where the data on each unit are just scalar observations Y_{hl}, say. That is, Y_{hl} is a vector of length $n=1$. In this section, we give brief statements of these generalizations without much justification. A more in-depth treatment of the general multivariate problem may be found in Johnson and Wichern (2002). First, consider the case of just $q=2$ groups. If the observations are just scalars rather than vectors, then we would be interested in the comparison of two scalar means μ_l and H_o would reduce to

$$H_o : \mu_1 = \mu_2 \text{ or } H_o : \mu_1 - \mu_2 = 0 \qquad (3.22)$$

Furthermore, the unknown covariance matrix Σ would reduce to a single scalar variance value, σ^2. Under our normality assumption, the standard test of H_o would be the two-sample t test. Because σ^2 is unknown, it must be estimated. This is accomplished by estimating

σ^2 based on the observations for each group and then "pooling" the result. That is, letting $\bar{Y}_{.l}$ denote the sample mean of the r_l observations y_{hl} for group l find the sample variance.

$$S_l^2 = (r_l - 1)^{-1} \sum_{h=1}^{r_l} (Y_{hl} - \bar{Y}_{.l})^2 \tag{3.23}$$

and construct the estimate of σ^2 from data in both group as the "weighted average"

$$S^2 = (r_1 + r_2 - 2)^{-1} \left\{ (r_1 - 1) S_1^2 + (r_2 - 1) S_2^2 \right\} \tag{3.24}$$

Now form the test statistic

$$t = \frac{\bar{Y}_{.1} - \bar{Y}_{.2}}{\sqrt{(r_1^{-1} + r_2^{-1}) S^2}} \tag{3.25}$$

The statistic t may be shown to have a student's t distribution with $r_1 + r_2 - 2$ degrees of freedom.

In multivariate case, the hypothesis is now

$$H_o : \mu_1 = \mu_2 \text{ or } H_o : \mu_1 - \mu_2 = 0 \tag{3.26}$$

A natural approach is to seek a multivariate analogue to the t test.

The analogue of the assumed common variance σ^2 is now the assumed common covariance matrix Σ which is of course unknown. We would like to estimate this matrix for each group and then "pool" the results.

In particular, we may calculate the pooled sample covariance matrix. If we collect the sample means $\bar{Y}_{.lj}$, $j=1, \ldots, n$ into a vector

$$\bar{Y}_l = \begin{pmatrix} \bar{y}_{l1} \\ . \\ . \\ . \\ \bar{y}_{ln} \end{pmatrix},$$

then the sample covariance matrix for group l is the (nxn) matrix.

$$\hat{\Sigma}_l = (r_1 - 1)^{-1} \sum_{h=1}^{r_l} (Y_{hl} - \bar{Y}_{.l})(Y_{hl} - \bar{Y}_{.l})' \tag{3.27}$$

We know that the sum in (3.27) is called a sum of squares and cross-products (SS & CP) matrix.

The overall pooled sample covariance, an estimator for Σ is then the "weighted average".

$$\hat{\Sigma} = (r_1 + r_2 - 2)^{-1} \left\{ (r_1 - 1)\hat{\Sigma}_1 + (r_2 - 1)\hat{\Sigma}_2 \right\} \tag{3.28}$$

The test statistics analogues to the (square of) the t statistic is known as Hotelling's T^2 statistic and is given by

$$T^2 = \left(r_1^{-1} + r_2^{-1}\right)^{-1}\left(\overline{Y}_{.1} - \overline{Y}_{.2}\right)'\hat{\Sigma}^{-1}\left(\overline{Y}_{.1} - \overline{Y}_{.2}\right) \tag{3.29}$$

It can be shown that

$$\frac{r_1 + r_2 - n - 1}{(r_1 + r_2 - 2)n} T^2 \sim F_{(n, r_1 + r_2 - n - 1)} \tag{3.30}$$

Thus, the test of H_o may be carried out at α level by comparing this version of T^2 to the appropriate α critical value.

Note that if n = 1, the multiplicative factor is equal to 1 and the statistic has an F distribution with 1 and r_1+r_2-2 degrees of freedom, which is just the square of the $t_{r_1 + r_2 - 2}$ distribution. That is, the multivariate test reduces to the scalar t test if the dimension of a data vector $n=1$.

It is worth noting that the hypothesis in (3.26) may be expressed in matrix form. Specifically, if we define ***M*** as *(2xn)* matrix whose rows are the transposed mean vectors μ_1' and μ_2',

$$\mathbf{M} = \begin{pmatrix} \mu_{11} & \cdots & \mu_{1n} \\ \mu_{21} & \cdots & \mu_{2n} \end{pmatrix}$$

It is clear that, defining C = (1,-1), we have

$$\mathbf{CM} = \left(\mu_{11} - \mu_{21}, \ldots, \mu_{1n} - \mu_{2n}\right) = \left(\mu_1 - \mu_2\right)'$$

Thus, we may express the hypothesis in the form

$$H_o\text{: } \mathbf{CMU = 0, U = I_n.} \tag{3.31}$$

3.3.3.3 One-way MANOVA

Just as the case of comparing 2 group means for scalar response may be generalized to $q>2$ groups using analysis of variance techniques, the multivariate analysis above also may be generalized.

Again, if the observations were just scalars, we would be interested in the comparison of *q* scalar means μ_l, l = 1,…, q, and H_o would reduce to

$$H_o : \mu_1 = \ldots = \mu_q \tag{3.32}$$

and again the unknown covariance matrix Σ would reduce to a single scalar variance value σ^2. Under the normality assumption, the standard test of H_o via one-way analysis of variance is based on the ratio of two estimators for σ^2. The following is the usual one-way analysis of variance; recall that $m = \sum_{l=1}^{q} r_l$ is the total number of units.

Table 3.2 ANOVA Table

Source	DF	SS	MS	F
Among Units	q-1	$SS_G = \sum_{l=1}^{q} r_l \left(\bar{Y}_{.l} - \bar{Y}_{..}\right)^2$	MS$_G$	MS$_G$/MS$_E$
Among-Unit Error	m-q	$SS_E = \sum_{h=1}^{r_l} \left(Y_{hl} - \bar{Y}_{.l}\right)^2$	MS$_E$	
Total	m-1	$\sum_{l=1}^{q} \left(Y_{hl} - \bar{Y}_{..}\right)^2$		

Note that the "error" sum of square SS$_E$ may be written as

$$SS_E = (r_1 - 1)S_1^2 + \ldots + (r_q - 1)S_q^2, \text{ and } S_l^2 = (r_1 - 1)^{-1} \sum_{h=1}^{r_l} \left(Y_{hl} - \bar{Y}_{.l}\right)^2 \qquad (3.33)$$

where S_l^2 is the sample variance for the l^{th} group, so that MS$_E$ has the interpretation as the pooled sample variance estimator for σ^2 across all q groups. MS$_G$ is an estimator for σ^2 based on deviations of the group means from the overall mean, and will overestimate σ^2 if the means are different. It may be shown that the ratio F has sampling distribution that is F with *(q-1)* and *(m-q)* degrees of freedom, so that the test is conducted at level α by comparing the calculated value of F to $F_{q-1,m-q,\alpha}$.

For the multivariate case, the hypothesis is now

$$H_o : \mu_1 = \ldots = \mu_q \qquad (3.34)$$

As in the case of $q=2$ groups above, the multivariate generalization involves the fact that is now an entire covariance matrix Σ to estimate rather than just a single variance. Consider the following analogue to the scalar one-way analysis of variance above. Let $\bar{Y}_{..j}$ be the sample mean of all observations across all units and groups for the j^{th} element and define the overall mean vector

$$\overline{Y}_{..} = \begin{pmatrix} \overline{Y}_{..1} \\ . \\ . \\ . \\ \overline{Y}_{..n} \end{pmatrix}$$

Table 3.3 MANOVA Table

Source	**DF**	**SS & CP**
Among Groups	q-1	$Q_H = \sum_{l=1}^{q} r_l (\overline{Y}_{.l} - \overline{Y}_{..})(\overline{Y}_{.l} - \overline{Y}_{..})'$
Among Unit Error	m-q	$Q_E = \sum_{l=1}^{q} (Y_{hl} - \overline{Y}_{.l})(Y_{hl} - \overline{Y}_{.l})'$
Total	m-1	$Q_H + Q_E = \sum_{l=1}^{q} \sum_{h=1}^{r_l} (Y_{hl} - \overline{Y}_{..})(Y_{hl} - \overline{Y}_{..})'$

Comparing the entries in this table to those in the scalar ANOVA table, we see that they appear to be multivariate generalizations. In particular, the entries are now matrices. Each may be viewed as an attempt to estimate Σ.

It can be verified that the among unit error sum of squares and cross products matrix Q_E may be written as

$$Q_E = (r_1 - 1)\hat{\Sigma}_1 + \ldots + (r_q - 1)\hat{\Sigma}_q, \tag{3.35}$$

where $\hat{\Sigma}_l$ the estimate (3.27) of Σ is based on the data vectors from group l. Thus, just as in the scalar case, this quantity divided by its degrees of freedom has the interpretation as a "pooled" estimate of Σ across groups.

Unfortunately, because these entries are matrices, it is no longer straightforward to construct a unique generalization of the F ratio that may be used to test H_0. Clearly, one would like to compare the "magnitude" of the SS&CP matrices Q_H and Q_E somehow, but there is no one way to do this. There are a number of statistics that have this interpretation.

a) **Wilks' Lambda**

The most commonly discussed statistic used and may be motivated formally as follows. In the scalar case, the F ratio is

$$\frac{SS_G/(q-1)}{SS_E/(m-q)} \tag{3.36}$$

Thus, in the scalar case, H_o is rejected when the ratio SS_G/SS_E is large. This is equivalent to rejecting for large values of $1+\left(\frac{SS_G}{SS_E}\right)$ or small values of

$$\frac{1}{1+\left(\frac{SS_G}{SS_E}\right)}=\frac{SS_E}{SS_G+SS_E}$$

For the multivariate problem, the Wilks' lambda statistic is the analogue of this quantity,

$$T_W=\frac{|Q_E|}{|Q_H+Q_E|} \tag{3.37}$$

Here, the determinant of each SS&CP matrix is taken, reducing the matrix to a single number. This number is often referred to as the generalized sample variance (Johnson & Wichren, 2002). One rejects H_o for small values of T_W.

b) **Lawley-Hotelling Trace**

This test statistic rejects H_o for large values of

$$T_{LH}=tr(Q_H Q_E^{-1}) \tag{3.38}$$

There are also another statistics: Pilla's trace and Roy's greatest root.

None of these approaches is superior to the others in general. In addition, all are equivalent to using the Hotelling's T^2 statistic in the case $q=2$.

It is possible in certain special cases to work out the exact sampling distribution of these statistics. As mentioned above, when $q=2$ and we are testing whether the two means are the same, all of these statistics may be shown to be the same and equivalent to conducting the test based on Hotelling's T^2 statistics.

When $n=1, 2$ and $q\geq 2$ or when $n\geq 1$ and $q=2, 3$ it is possible to show that certain functions of T_W have an F sampling distribution and this may be used to conduct the test exactly.

3.3.4 Mixed Model Approach to Repeated Measures Analysis

In the previous two sections, two sets of results were discussed: Univariate (ANOVA) and Multivariate (MANOVA) results. These results are based on different assumptions about the structure of the covariance of the scores across time. The univariate model uses a structure called compound symmetry. There is a single variance for all of the trials, and there is a single covariance for each of the pairs of trials. The multivariate model uses a structure called unstructured. Each trial has its own variance and each pair of trials has its own covariance.

But these are not the only possible covariance structures and also they have serious drawbacks. The mixed model allows to select among a wide variety of covariance structures allowing us to choose the appropriate covariance structure for our data.

This approach simplifies and unifies many common statistical analyses, including those involving repeated measures, repeated measures random effects and random coefficients. The basic assumption is that the data are linearly related to unobserved multivariate normal random variables.

3.3.4.1 Matrix Notation

Suppose that we observe n data points $y_1,\ldots,y_n$ and that we want to explain them using n values for each of p explanatory variables $x_{11}, \ldots, x_{1p}, x_{21},\ldots, x_{2p},\ldots,x_{n1},\ldots,x_{np}$. The x_{ij} values may be either regression type continuous variables or dummy variables indicating class membership. The standard linear model for this set up is

$$y_i = \sum_{j=1}^{p} x_{ij}\beta_j + e_i \qquad \text{i=1,..., n} \tag{3.39}$$

where $\beta_1,\ldots,\beta_p$ are unknown fixed effects parameters to be estimated and $e_1,\ldots,e_n$ are unknown independent and identically distributed normal (Gaussian) random variables with mean 0 and variance σ^2. The preceding equations can be written simultaneously using vectors and a matrix, as follows:

$$\begin{bmatrix} y_1 \\ y_2 \\ \cdot \\ \cdot \\ \cdot \\ y_n \end{bmatrix} = \begin{bmatrix} x_{11} & x_{12} & \dots & x_{1p} \\ x_{21} & x_{22} & \dots & x_{2p} \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ x_{n1} & x_{n2} & \dots & x_{np} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \cdot \\ \cdot \\ \cdot \\ \beta_p \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ \cdot \\ \cdot \\ \cdot \\ e_n \end{bmatrix}$$

For convenience, simplicity and extendibility, this entire system is written as

$$Y = X\beta + e \tag{3.40}$$

where Y denotes the vector of observed y_i's, X is the known matrix of x_{ij}'s, β is the unknown fixed effects parameter vector, and e is the unobserved vector of independent and identically distributed Gaussian random errors.

3.3.4.2 Formulation of the Mixed Model

The following discussion on mixed models is taken from SAS for Mixed Models by Littel et al. (2006).

The previous general linear model is certainly a useful one (Searle, 1971), and it is the one fitted by the GLM procedure. However, many times the distributional assumption about ε is too restrictive. The mixed model extends the general linear model by allowing a more flexible specification of the covariance matrix of e. In other words, it allows for both correlation and heterogeneous variances, although we still assume normality.

The mixed model is written as

$$Y = X\beta + Z\gamma + e \tag{3.41}$$

where everything is the same as in the general linear model except for the addition of the known design matrix, Z, and the vector of unknown random effects parameters, γ. The matrix Z can contain either continuous or dummy variables, just like X. The name mixed model comes from the fact that the model contains both fixed effects parameters, β, and random effects parameters, γ.

A key assumption in the foregoing analysis is that γ and e are normally distributed with

$$E\begin{bmatrix} \gamma \\ e \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix} \text{ and } Var\begin{bmatrix} \gamma \\ e \end{bmatrix} = \begin{bmatrix} G & 0 \\ 0 & R \end{bmatrix}$$

The variance of Y is, therefore, **V** = **ZGZ'** + **R**. We can model **V** by setting up the design matrix **Z** and by specifying covariance structures for **G** and **R**.

This is a general specification of the mixed model in contrast to many texts and articles that discuss only simple random effects. Simple random effects are a special case of the general specification with **Z** containing dummy variables, **G** containing variance components in a diagonal structure, and $R = \sigma^2 I_n$, where I_n denotes the n x n identity matrix. The general linear model is a further special case with $Z = 0$ and $R = \sigma^2 I_n$

Analysis of repeated measures data requires special attention to the covariance structures due to the sequential nature of the data on each subject. Ignoring the covariance issues may result in incorrect conclusions from the statistical analyses. Avoiding the issues may result in inefficient analysis which is tantamount to wasting data. The general linear mixed model allows the capability to address the issue directly by modeling the covariance structure.

There are two basic concepts in performing a repeated measures analysis using mixed model methodology. The first step is to model the covariance structure. The second step is to test the main effects and interaction effects using the modeled covariance structure.

The following are the most commonly known covariance structures in repeated measures designs:
Compound Symmetry Structure has two unknown parameters and has the form

$$\begin{bmatrix} \sigma_1^2+\sigma^2 & \sigma_1^2 & \sigma_1^2 & & & & \\ & \sigma_1^2+\sigma^2 & \sigma_1^2 & & & & \\ & & \sigma_1^2+\sigma^2 & & & & \\ & & & \ddots & & & \\ & & & & \sigma_1^2+\sigma^2 & \sigma_1^2 & \sigma_1^2 \\ & & & & & \sigma_1^2+\sigma^2 & \sigma_1^2 \\ & & & & & & \sigma_1^2+\sigma^2 \end{bmatrix}.$$

The *Huynh-Feldt* or *type H* has $t+1$ unknown parameters in the following form

$$\begin{bmatrix} \sigma_1^2 & \frac{\sigma_1^2+\sigma_2^2}{2}-\lambda & \frac{\sigma_1^2+\sigma_3^2}{2}-\lambda & \cdots & \frac{\sigma_1^2+\sigma_{t-1}^2}{2}-\lambda & \frac{\sigma_1^2+\sigma_t^2}{2}-\lambda \\ & \sigma_2^2 & \cdot & & & \\ & & \cdot & & \cdot & \cdot \\ & & & \cdot & \cdot & \cdot \\ & & & \cdot & \cdot & \cdot \\ & & & & \sigma_{t-1}^2 & \frac{\sigma_{t-1}^2+\sigma_t^2}{2}-\lambda \\ & & & & & \sigma_t^2 \end{bmatrix}$$

Autoregressive homogeneous variance structure has the form

$$\begin{bmatrix} \sigma^2 & \rho\sigma^2 & \rho^2\sigma^2 & \cdots & \rho^{t-1}\sigma^2 \\ & \sigma^2 & \rho\sigma^2 & \cdots & \rho^{t-2}\sigma^2 \\ & & \sigma^2 & & \cdot \\ & & & \cdot & \cdot \\ & & & \cdot & \cdot \\ & & & & \sigma^2 \end{bmatrix}$$

Autoregressive heterogeneous variance structure

$$\begin{bmatrix} \sigma_1^2 & \rho\sigma^2 & \rho^2\sigma^2 & \cdots & \rho^{t-1}\sigma^2 \\ & \sigma_2^2 & \rho\sigma^2 & \cdots & \rho^{t-1}\sigma^2 \\ & & \cdot & & \cdot \\ & & & \cdot & \cdot \\ & & & & \cdot \\ & & & & \sigma_t^2 \end{bmatrix}$$

The *unstructured matrix* is the most general form possible and has $\frac{t(t+1)}{2}$ unknown parameters. Where t is number of levels of the within subject factor.

$$\begin{bmatrix} \sigma_{11} & \sigma_{12} & \cdot & \cdot & \cdot & \sigma_{1t} \\ & \sigma_{22} & \cdot & \cdot & \cdot & \sigma_{2t} \\ & & \cdot & & & \cdot \\ & & & \cdot & & \cdot \\ & & & & \cdot & \cdot \\ & & & & & \sigma_{tt} \end{bmatrix}$$

3.3.4.3 Estimating G and R in the Mixed Model

Estimation is more difficult in the mixed model than in the general linear model. Not only do we have β as in the general linear model, but we have unknown parameters in γ, **G**, and **R** as well. Least squares is no longer the best method. Generalized least squares (GLS) is more appropriate, minimizing

$$(Y - X\beta)'V^{-1}(Y - X\beta) \tag{3.42}$$

However, it requires knowledge of **V** and, therefore, knowledge of **G** and **R**. Lacking such information, one approach is to use estimated GLS, in which you insert some reasonable estimate for **V** into the minimization problem. The goal thus becomes finding a reasonable estimate of **G** and **R** (Littel et al., 2006).

In many situations, the best approach is to use likelihood-based methods, exploiting the assumption that γ and e are normally distributed. The two likelihood-based methods are maximum likelihood (ML) and restricted/residual maximum likelihood (REML). A favorable theoretical property of ML and REML is that they accommodate data that are missing at random (Rubin, 1976).

SAS mixed procedure constructs an objective function associated with ML or REML and maximizes it over all unknown parameters. Using calculus, it is possible to reduce the maximization problem to one in the parameters of **G** and **R**. The corresponding log-likelihood functions are as follows:

$$\text{ML: } l(G,R) = -\frac{1}{2}\log|V| - \frac{1}{2}r'V^{-1}r - \frac{n}{2}\log(2\pi) \tag{3.43}$$

$$\text{REML: } l_R(G,R) = -\frac{1}{2}\log|V| - \frac{1}{2}\log|X'V^{-1}X| - \frac{1}{2}r'V^{-1}r - \frac{n-p}{2}\log(2\pi) \tag{3.44}$$

where $r = Y - X\left(X'V^{-1}X\right)^{-}X'V^{-1}Y$ and $p = rank(X)$

To maximize this objective function we use the mixed model equations

$$\begin{bmatrix} X'\hat{R}^{-1}X & X'\hat{R}^{-1}Z \\ Z'\hat{R}^{-1}X & Z'\hat{R}^{-1}Z + \hat{G}^{-1} \end{bmatrix} \begin{bmatrix} \hat{\beta} \\ \hat{\gamma} \end{bmatrix} = \begin{bmatrix} X'\hat{R}^{-1}Y \\ Z'\hat{R}^{-1}Y \end{bmatrix}$$

Thus, the solutions can be written as

$$\hat{\beta} = \left(X'\hat{V}^{-1}X\right)^{-} X'\hat{V}^{-1}Y \text{ and } \hat{\gamma} = \hat{G}Z'\hat{V}^{-1}\left(Y - X\hat{\beta}\right) \tag{3.45}$$

Note that the mixed model equations are extended normal equations and that the preceding expression assumes that $\hat{G}$ is nonsingular. For the extreme case when the eigenvalues of $\hat{G}$ are very large, $\hat{G}^{-1}$ contributes very little to the equations and $\hat{\gamma}$ is close to what it would be if γ actually contained fixed effects parameters. On the other hand, when the eigenvalues of $\hat{G}$ are very small, $\hat{G}^{-1}$ dominates the equations and $\hat{\gamma}$ is close to 0. For intermediate cases, $\hat{G}^{-1}$ can be viewed as shrinking the fixed effects estimates of γ towards 0 (Littel et al., 2006).

If $\hat{G}$ is singular, then the mixed model equations are modified (Henderson, 1984) as follows:

$$\begin{bmatrix} X'\hat{R}^{-1}X & X'\hat{R}^{-1}Z\hat{L} \\ \hat{L}'Z\hat{R}^{-1}X & \hat{L}'Z\hat{R}^{-1}Z\hat{L}+I \end{bmatrix}\begin{bmatrix} \hat{\beta} \\ \hat{\gamma} \end{bmatrix}=\begin{bmatrix} X'\hat{R}^{-1}Y \\ \hat{L}'Z'\hat{R}^{-1}Y \end{bmatrix}$$

Where $\hat{L}$ is the lower-triangular Cholesky root of $\hat{G}$, satisfying $\hat{G}=\hat{L}\hat{L}'$. Both $\hat{\gamma}$ and a generalized inverse of the left-hand-side coefficient matrix are then transformed using $\hat{L}$ to determine $\hat{\gamma}$.

3.3.4.4 Model Selection

The previous section on estimation assumes the specification of a mixed model in terms of **X, Z, G** and **R**. Even though **X** and **Z** have known elements, their specific form and construction is flexible, and several possibilities may present themselves for a particular data set. Likewise, several different covariance structures for **G** and **R** might be reasonable.

Model selection can be done in two ways. First subject matter considerations and objectives are of great importance when selecting a model (Diggle, 1988; Lindsey, 1993). Second, when the data themselves are used for guidance, many of the graphical methods and diagnostics appropriate for the general linear model extend to the mixed model setting as well. Finally, a likelihood-based approach to the mixed model provides several statistical measures for model adequacy as well. The most common of these are the likelihood ratio test and Akaike's and Schwarz's Bayesian Information Criteria (AIC and BIC) (Bozdogan, 1987; Wolfinger, 1993)

3.3.4.5 Inference and Test Statistics

For inferences concerning the covariance parameters in our model, one can use likelihood–based statistics. One common likelihood-based statistic is the Wald Z, which is computed as the parameter estimate divided by its asymptotic standard error. The asymptotic standard errors are computed from the inverse of the second derivative matrix of the likelihood with respect to each of the covariance parameters. The Wald Z is valid for large samples, but it can be unreliable for small data sets and for parameters such as variance components, which are known to have a skewed or bound sampling distribution.

A better alternative is the likelihood ratio χ^2. This statistic compares two covariance models, one a special case of the other. The likelihood ratio χ^2- statistic is computed by subtracting the corresponding values of REML loglikelihood and multiplying by -2. Either ML or REML can be used to construct this statistic, which tests whether the full model is necessary beyond the reduced model.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Diagnostic Checking

The data used in the study are repeated measurements of the pseudostem circumference of *enset* varieties. In order to apply the univariate analysis of variance, it is assumed that the error terms are identically, independently and normally distributed with mean zero and constant variance (σ^2). Hence, whether the data fulfill these assumptions is checked. The residual analysis is carried out using SAS and the results are displayed in Appendix B (one output and two graphs).

The assumption that the error terms are distributed with zero mean is tested using the Student's t statistic and found not significantly different from zero ($p = 0.9995$). The normality assumption is tested using Shapiro-Wilk Statistic. The null hypothesis is not rejected ($p=0.3666$). Hence, we conclude that normality is satisfied. Normality is also justified using the normal probability plot and histogram which are straight line and bell shaped curves, respectively. The studentized residual plot against time depicts the homogeneity of error variances (constant variance) since no pattern is observed and most of the points in the plot lie between -2 and 2 and no pattern is observed (Appendix B). Hence, the assumptions of normality, zero mean and homogeneity of variance of the error term is met for the *enset* data.

4.2 Summary Statistics

Summary statistics is the first approach which is recommended for analysis of repeated measures data as a descriptive approach for its simplicity and possible indicator of the presence or absence of main and interaction effects. It is safe in the sense that the significance tests will tend to be conservative. The results of this method are used as indicators of significance tests using the most complicated statistical techniques. All the information on each individual is reduced to just one single measure which is believed, in the context of the particular experiment, to be a useful summary of the responses. For example, it may be decided that for an individual subject, the mean of the responses at all the time points after the start of the treatment is a sensible representation of the individual's overall response to treatment. In the *enset* data, the summary measures are the mean of pseudostem circumference of the four replications of varieties at each time of measurement. Table 4.1 shows the mean of the *enset* varieties at each time of measurement.

Table 4.1 Least Squares Means of the Pseudostem Circumference

Variety	Mean(cm) at Time 1	Mean(cm) at Time 2	Mean(cm) at Time 3	Mean(cm) at Time 4	Overall Mean(cm)
1	0.3100	0.4225	0.6700	0.7875	**0.5475**
2	0.6175	0.5350	0.9825	0.7625	**0.7244**
3	0.6175	0.6375	1.0750	0.7250	**0.7638**
4	0.6350	0.6200	1.0600	0.7375	**0.7631**
5	0.5400	0.4975	0.9875	0.7000	**0.6813**
6	0.4100	0.6725	0.9225	0.7125	**0.6794**
7	0.3650	0.2700	0.8625	0.7125	**0.5525**
8	0.5150	0.4425	0.7600	0.7375	**0.6138**
9	0.4900	0.4925	1.0500	1.1250	**0.7894**
10	0.6575	0.6325	1.1250	1.1125	**0.8819**
11	0.4925	0.5600	0.9250	1.1250	**0.7756**
12	0.5475	0.5425	1.0075	0.9250	**0.7556**
13	0.4225	0.5725	0.7675	0.7500	**0.6281**
14	0.5050	0.5825	1.1500	1.1750	**0.8531**
15	0.5400	0.7125	1.0800	1.1500	**0.8706**
16	0.3375	0.4900	0.6750	0.8000	**0.5756**
17	0.6725	0.5900	1.1000	1.0200	**0.8456**
18	0.6750	0.5775	1.0375	1.0000	**0.8225**
19	0.4525	0.8050	0.9000	0.8750	**0.7581**
20	0.4750	0.6700	0.6625	0.7325	**0.6350**
21	0.4600	0.5900	0.7750	1.1000	**0.7313**
22	0.4400	0.5500	0.5925	0.6500	**0.5581**
23	0.5250	0.2675	0.6625	0.7425	**0.5494**
24	0.5950	0.4725	0.8150	0.8100	**0.6731**
25	0.5225	0.3175	0.7500	0.8250	**0.6038**
26	0.4850	0.3900	0.6000	0.5500	**0.5063**

Table 4.1 (Continued)

27	0.5350	0.5375	0.6875	0.7750	**0.6338**
28	0.6375	0.3575	0.6750	0.6875	**0.5894**
29	0.7875	0.5375	0.8525	0.7775	**0.7388**
30	0.7325	0.4925	0.8425	1.0875	**0.7888**
31	0.5250	0.5800	0.8000	1.1625	**0.7669**
32	0.6300	0.6350	0.8375	1.1800	**0.8206**
33	0.6175	0.5350	0.8875	0.9825	**0.7556**

From Table 4.1, it can be seen that mean pseudostem circumference of some varieties shows an increase while others show a decrease in mean circumference as one goes from time 1 to time 2. Moreover, as we go from time 2 to time 3 mean pseudostem circumference of all varieties except variety 20 show an increase. We also observe a difference in mean pseudostem circumference among the various varieties (*enset* clones). The rate of increase or decrease in pseudostem circumference as one goes from left to right is also different among varieties. The standard ANOVA tests that will be considered in the next sections help whether these differences are statistically significant or not.

Another summary measure commonly used in repeated measurements is to plot the mean of the response variable against time. Figure 1 shows the pseudostem circumference mean plot of the *enset* varieties against time.

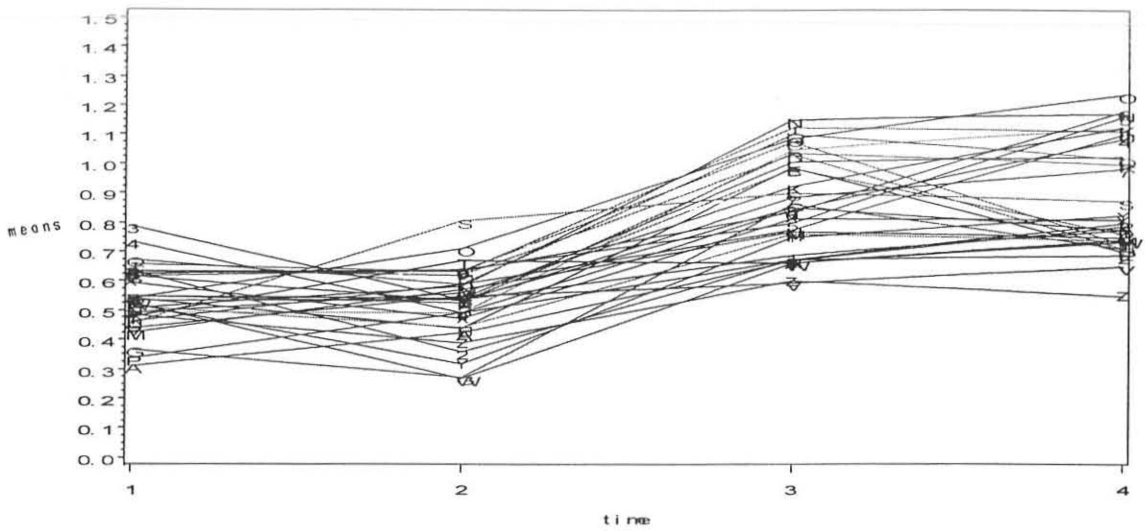

Figure 1 Plot of mean of different pseudostem circumference varieties against time.

The fact that the lines do not coincide shows that the pseudostem circumference means of the *enset* varieties are different. When we look at the overall tendency of graph, it seems that it is increasing as we go from left to right. The lines on the graph are not parallel and they cross one another showing that there is an interaction effect between variety and time. Looking closely at particular varieties, variety S increases rapidly from time 1 to time 2 and then decreases slowly from time 2 to time 3 and again from time 3 to time 4. This indicates that pseudostem circumference of variety S grows faster at younger stage and shrinks at maturity. But variety G decreases from time 1 to time 2, then increases from time 2 to time 3 and again decreases from time 3 to time 4 which indicates fluctuation of pseudostem circumference of variety G at successive time points.

It is always helpful, if possible, to plot the data to give a broad indication of what is happening. The raw data could be plotted against time. All the information could be retained in one diagram, so that every response for all individuals is shown. However, if there are a large number of individuals, this can produce a confusing diagram which fails to achieve its aim of demonstrating trends and relationships. In such instances, a separate diagram can be produced for each treatment group. Alternatively, separate graphs of the responses against time for each individual can be drawn; each should be drawn on the same scale, perhaps grouping the graphs for each treatment in a grid. Often there are so many graphs that become unwieldy to include them all in a paper.

Then, just representative ones which are believed to illustrate particular types of response structures may be included. The subjectivity of this approach may be open to criticism, so the choices should be justified.

The descriptive methods considered in this section are used as a preliminary analysis since they only help as an indication for the presence of main and interaction effects. But they do not show whether these effects are statistically significant or not. Hence, a more formal alternative approaches are presented in sections that follow.

4.3 Univariate Analysis of Variance (ANOVA)

The procedure known as Analysis of Variance or ANOVA is used to test the hypotheses concerning means when we have several populations. ANOVA is a general technique that can be used to test the hypothesis that the means among two or more groups are equal. Here, means of the various *enset* varieties are compared. Assuming that all the ANOVA assumptions are satisfied, the standard analysis of variance is performed. The following is ANOVA table for the *enset* data:

Table 4.2: ANOVA Table of *Enset* Data

Source	DF	Sum of Squares	Mean Squares	F Value	P-Value
Variety	32	6.02992538	0.18843517	20.33	< 0.0001
Plant(Variety)	99	1.12943125	0.01140840	1.23	0.0947
Time	3	15.27342936	5.09114312	549.29	< 0.0001
Variety × Time	96	5.63610189	0.05870939	6.33	< 0.0001
Error	297	2.75279375	0.00926867		
Corrected Total	**527**	**30.82168163**			

The standard ANOVA (Table 4.2) shows that the variety means are significantly different from one another ($p < 0.0001$). Further more, the variability of plants within the same variety are not significantly different ($p = 0.0947$).

The ANOVA table also shows that the mean of pseudostem circumference is significantly different at different times. That is, time has significant effect on pseudostem circumferences ($p < 0.0001$).In fact, the significance is as expected since the plant is growing and hence the pseudostem circumference keeps increasing as time goes on until the plant reaches maturity.

As can be seen on the fourth row of Table 4.2, there is also significant variety by time interaction ($p < 0.0001$). Hence, the hypothesis of no variety by time interaction is rejected and concluded that there is significant interaction between variety and time. In other words, time affects the varieties differently.

However, all the above ANOVA tests are valid if and only if the underlying assumptions of the repeated measures ANOVA are satisfied. Repeated measures ANOVA carries the standard set of assumptions associated with an ordinary analysis of variance, extended to the matrix case: multivariate normality, homogeneity of covariance matrices, and independence. The assumptions of homogeneity of error variances and normality are checked and found to be satisfied in diagnostic checking section (Section 4.1) and the assumption of independence has not yet been checked. Responses measured on the same subject are correlated because they contain a common contribution from the subject. Moreover, measures on the same subject close in time tend to be more highly correlated than measures far apart in time. Repeated measures ANOVA is robust to violations of the first two assumptions. But violations of independence produce a non normal distribution of the residuals which result in invalid F ratios. Therefore, in addition to the assumptions already checked, the univariate approach for testing the within subject effects requires the assumption of sphericity of the transformed variables (i.e., the circularity of the original variables). The assumption of sphericity is tested using the transformed dependent variables. The original variables representing each trial are transformed according to a set of orthogonal contrasts. The choice of transformation does not affect the outcome of the test, as long as the transformation matrix is orthonormal. Table 4.3 presents test for sphericity.

Table 4.3: Sphericity Tests

Variables	DF	Mauchley's Criterion	Chi - Square	Pr > Chi - Square
Transformed Variates	5	0.8742027	13.138068	0.0221
Orthogonal Components	5	0.8742027	13.138068	0.0221

Although the output shows two separate tests of sphericity, the one of interest is the orthogonal components test, which is the test of sphericity applied to the common covariance matrix of the transformed within subject variables. Mauchley's sphericity test examines the form of the common covariance matrix. A spherical matrix has equal variances and has covariances equal to zero. The common covariance matrix of the transformed within-subject variables must be spherical; otherwise the F tests and associated p values for the univariate approach for testing within-subject hypotheses are invalid. If the chi-square approximation has an associated p value less than the selected alpha level, the sphericity assumption has been violated. The chi-square approximation for this test is 13.138068 with 5 df and an associated probability of 0.0221. Since this value is less than the tabulated chi-square value at 0.05, it seems that the data does not meet the assumption of sphericity but if we set our alpha to smaller level (0.01), the sphericity assumption will not be rejected. Hence, there is no strong evidence that the transformed variable deviates from sphericity assumption. This test will help to decide which method to use and whether the results should be adjusted. The univairate method will have more power but it is appropriate only when the sphericity assumption is not violated. If the sphericity assumption is violated, then in most situations we are better off staying with the multivariate method. An alternative to using the multivariate approach is to adjust the F values involving a within-subject effect tests for degrees of freedom. The two correction factors for degrees of freedom are the Greenhouse-Geisser Epsilon (G-G) and the Huynh-Feldt Epsilon (H-F). Generally, the H-F correction factor is used because the G-G correction factor has been shown to be too conservative (Huynh and Feldt, 1976). The following are the between and within subject effect tests for the *enset* data.

4.3.1 Between-Subject Effect Tests

Between-subject effect tests test the variability among various groups. In the *enset* data, these tests involve comparing the pseudostem circumference means of *enset* plants which belong to different varieties. In other words, we test whether the various *enset* varieties yield the same psudostem circumference.

Table 4.4 Repeated Measures Analysis of Variance Tests of Hypothesis for Between-variety Effects

Source	**DF**	**Sum of Squares**	**Mean Squares**	**F Value**	**Pr >Value**
Variety	32	6.02992538	0.18843517	16.52	< 0.0001
Error	99	1.12943125	0.01140840	-	-

In Table 4.4, the row labeled "Variety" reports the sum of squares, degrees of freedom, and mean square for variety. The row also reports the F value and associated p value for the hypothesis test of the variety difference. With a p value less than 0.0001, there is a statistically significant variety effect. Therefore, this is an indication that a statistically significant difference exists between varieties on their overall pseudostem circumference. From this result it is evident that different *enset* varieties give rise to different pseudostem circumference and hence different yields and this can help the researchers to select best yielding variety.

The row labeled "Error" reports the within-cell sum of squares, degrees of freedom, and mean square, for the between-subject hypothesis.

4.3.2 Within-Subject Effect Tests

These tests are used to compare means belonging to the same group at different levels and test the presence of interaction between groups and levels of groups. In the problem of *enset* data, the means of pseudostem circumference of *enset* plant of the same variety at different time points are compared whether they yield the same mean and the presence of variety by time interaction are tested.

When there is severe deviation from the sphericity assumption, the F and p values in the ANOVA table are subject to adjustment. With non-spherical data, it is necessary to use adjusted results for the univariate tests. That is, the F results should be adjusted for their degrees of freedom.

Table 4.5 Repeated Measures Analysis of Variance Univariate Tests of Hypotheses for Within-Subject Effects

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F	G-G	H-F
Time	3	15.27342936	5.09114312	549.29	<.0001	<.0001	<.0001
Time × Variety	96	5.63610189	0.05870939	6.33	<.0001	<.0001	<.0001
Error	297	2.75279375	0.00926867				

Greenhouse - Geisser Epsilon: 0.9277, Huynh-Feldt Epsilon: 1.2658

The within-subject test is shown in Table 4.5. The corrected univariate p values appear under the G-G and H-F headers. Both the G-G and H-F p values indicate that there are significant differences among measurements at different times and also significant interaction between variety and time. (G-G and H-F adj. p values < 0.0001 for time and variety × time interaction).

The sphericity test results using Mauchley's criterion (Section 4.3) confirm that there is no strong evidence to conclude that the data deviates from the sphericity assumption. Moreover, Greenhouse-Geisser and Huynh-Feldt Epsilon estimates are 0.9277 and 1.2658, respectively. Both epsilons are very close to 1. Hence, no need of adjustment for degrees of freedom of the F statistic. These are the reasons why F values are not adjusted and hence the unadjusted p values are the same as the adjusted ones (Table 4.5).

4.4 Multivariate Approach to Within-Subject Tests

An alternative method to the adjusted degrees of freedom when the sphericity assumption is violated in univariate tests is using the multivariate approach. Unlike the univariate approach, it does not require the sphericity assumption. That is, the covariance structure can take any form. MANOVA analysis method regards the observations at different time points as separate variables. It gives reliable results for large sample size. Table 4.6 provides the within-subject effect tests of the *enset* data using multivariate analysis.

Table 4.6 MANOVA Test Criteria and Exact F Statistics for the Hypothesis of No Time Effect

Statistic	Value	F value	Num DF	Den DF	Pr >F
Wilks' Lambda	0.05277364	580.35	3	97	< 0.0001
Pillai's Trace	0.94722636	580.35	3	97	< 0.0001
Hotelling-Lawley Trace	17.94885308	580.35	3	97	< 0.0001
Roy's Greatest Root	17.94885308	580.35	3	97	< 0.0001

H = SS & CP Matrix, E = Error SSCP Matrix, S = 1, M=0.5, N = 47.5

Num DF= Numerator degrees of freedom

Den DF=Denominator degrees of freedom

The first multivariate test of a within-subject effect is the within-subject main effect test. It examines changes in pseudostem circumference as a function of time. The null hypothesis is that the mean pseudustem circumference does not change across different time points. The four rows of Table 4.6 report a separate multivariate test statistic (Wilks', Pillais', Hotelling's and Roy's). The Wilks' test is commonly used. The three values S, M and N are the degrees of freedom for the multivariate statistics. For statistics like Wilks' Lambda, three degrees of freedom are required to determine a critical value. The values of these multivariate statistics are converted to F values. In some cases, the converted F and its degrees of freedom are approximations.

Since the ratio for this hypothesis is very large [F (3, 97) = 580.35, $p < 0.0001$], we can confidently conclude that the pseudostem circumference changes with time.

The second multivariate test of a within-subject effect is the variety by time interaction effect test. The MANOVA table for this test is displayed in Table 4.7:

Table 4.7 MANOVA Test Criteria and F Approximations for the Hypothesis of No Variety × Time Effect.

Statistic	Value	F value	Num DF	Den DF	Pr>F
Wilk's Lambda	0.03321493	6.43	96	291.27	<.0001
Pillai's Trace	2.00827699	6.26	96	297	<.0001
Hotelling-Lawley Trace	6.62519123	6.61	96	252.7	<.0001
Roy's Greatest Root	3.36549486	10.41	96	99	<.0001

H = SS & CP Matrix for time x variety, E = Error SS & CP Matrix, S = 3, M=14, N=47.5

Note: F statistic for Roy's Greatest Root is an upper bound.

In this case, using Wilks' Lambda statistic, the F value associated with these multivariate tests of interaction is high; hence, the associated p value is low [F (96, 291.27) = 6.43, $p < 0.0001$].

Hence, we can conclude that there is significant interaction effect between variety and time. In other words, the change in mean pseudostem circumference across time depends upon variety. That is, different *enset* varieties do not have the same growth rates and a variety has different growth rates at different stages of growth.

4.5 Mixed Model Approach to Repeated Measures Analysis

The methods followed to analyze the *enset* data using the general linear model so far do not clearly give the form of variance-covariance structure of the data. In order to compare and select among the various variance-covariance structures, the mixed model would be used to find out the possible structures that the data might have. The possible structures are: compound symmetry, Huynh-Feldt

(Type H), autoregressive process of order 1 [AR(1)] and unstructured forms. The model is fitted using SAS mixed procedures. The results are given in the following Tables.

Table 4.8: Levelization Results

```
                          The Mixed Procedure
                        Class Level Information
              Class          Levels            Values
              Variety          33            1 2 3 4 5 6 7 8 9 10 11 12 13
                                             14 15 16 17 18 19 20 21 22 23
                                             24 25 26 27 28 29 30 31 32 33
              Plant             4              1 2 3 4
              Time              4              1 2 3 4
```

Next, the REML Estimation History and covariance parameter estimate tables are displayed in Table 4.9.

Table 4.9: REML Estimation Results

TYPE =CS:

```
                          REML Iteration History
        Iteration        Evaluation          Objective          Criterion
            0                1            - 524.71211031
            1                1            - 526.36838926       0.00000000
          Convergence criteria met.
                   Covariance Parameter Estimates (REML)
    Covariance Parameter    Estimate      Standard Error      Z        Pr > /Z/
    CS                      0.000535       0.000448          1.19      0.2322
    Residual                0.009269       0.000761         12.19    < 0.0001
```

TYPE =HF:

```
                          REML Iteration History
        Iteration      Evaluation        Objective         Criterion
            0               1            - 524.71211031
            1               2              - 545.50855003    0.00078456
            2               1              - 546.02236303    0.00001343
            3               1              - 546.03103881    0.00000001
         Convergence criteria met.
                   Covariance Parameter Estimates (REML)
    Covariance Parameter   Estimate     Standard Error      Z       Pr > /Z/
    Var (1)                0.006350     0.000905           7.01    < 0.0001
    Var (2)                0.01028      0.001150           8.93    < 0.0001
    Var (3)                0.009922     0.001169           8.49    < 0.0001
    Var (4)                0.01306      0.001349           9.68    < 0.0001
    HF                     0.009269     0.000761          12.19    < 0.0001
```

TYPE = AR (1):

```
                          REML Iteration History
        Iteration      Evaluation        Objective         Criterion
            0               1            - 524.71211031
            1               2              - 524.89089557    0.00000000
         Convergence criteria met.
                   Covariance Parameter Estimates (REML)
    Covariance Parameter   Estimate     Standard Error      Z       Pr > /Z/
    AR (1)                 0.02455       0.05804           0.42    < 0.6723
    Residual               0.009804      0.000697         14.06    < 0.0001
```

TYPE = UNS:

```
                         REML Iteration History
        Iteration        Evaluation        Objective          Criterion
            0                1          - 524.71211031
            1                1          - 559.34089921       0.00000000
        Convergence criteria met.
                   Covariance Parameter Estimates (REML)
Covariance Parameter   Estimate     Standard Error      Z         Pr > /Z/
      UN(1,1)          0.004889       0.000695         7.04       < 0.0001
      UN(2,1)         - 0.00001       0.000720        - 0.02        0.9849
      UN(2,2)          0.01050        0.001493         7.04       < 0.0001
      UN(3,1)          0.000223       0.000669         0.33         0.7393
      UN(3,2)         - 0.00049       0.000982        - 0.50        0.6147
      UN(3,3)          0.009063       0.001288         7.04       < 0.0001
      UN(4,1)          0.001590       0.000869         1.83         0.0672
      UN(4,2)          0.000676       0.001253         0.54         0.5896
      UN(4,3)          0.001229       0.001169         1.05         0.2932
      UN(4,4)          0.01476        0.002098         7.04       < 0.0001
```

The restricted maximum likelihood (REML) is used to estimate all unknown variance-covariance parameters (Jennrich and Schluchter, 1986). Table 4.9 displays the REML estimates of the four covariance structures.

The compound symmetry has two unknown parameters and has the following form:

$$\begin{bmatrix} \sigma^2+\sigma_1 & \sigma_1 & \sigma_1 & \sigma_1 \\ & \sigma^2+\sigma_1 & \sigma_1 & \sigma_1 \\ & & \sigma^2+\sigma_1 & \sigma_1 \\ & & & \sigma^2+\sigma_1 \end{bmatrix}$$

Here, the REML estimate of σ_1 is 0.000535 and that for σ^2 is 0.009269. (Table 4.9: Type=CS)

The Huynh-Feldt or Type H structure has five unknown parameters in the following form:

$$\begin{bmatrix} \sigma_1 & \frac{\sigma_1+\sigma_2}{2}-\lambda & \frac{\sigma_1+\sigma_3}{2}-\lambda & \frac{\sigma_1+\sigma_4}{2}-\lambda \\ & \sigma_2 & \frac{\sigma_2+\sigma_3}{2}-\lambda & \frac{\sigma_2+\sigma_4}{2}-\lambda \\ & & \sigma_3 & \frac{\sigma_3+\sigma_4}{2}-\lambda \\ & & & \sigma_4 \end{bmatrix}$$

The REML estimates for σ_1 to σ_4 are labeled as var(1) to var(4) and that for λ is labeled as HF. (Table 4.9: Type=HF)

The autoregressive structure has two parameters in the following form:

$$\begin{bmatrix} \sigma^2 & \sigma^2\rho & \sigma^2\rho^2 & \sigma^2\rho^3 \\ & \sigma^2 & \sigma^2\rho & \sigma^2\rho^2 \\ & & \sigma^2 & \sigma^2\rho \\ & & & \sigma^2 \end{bmatrix}$$

The REML estimates for σ^2 is 0.02455 and that for ρ is 0.009804. (Table 4.9: Type=AR(1))

The unstructured matrix is the most general form possible and has ten unknown parameters.

$$\begin{bmatrix} \sigma_{11} & \sigma_{12} & \sigma_{13} & \sigma_{14} \\ & \sigma_{22} & \sigma_{23} & \sigma_{24} \\ & & \sigma_{33} & \sigma_{34} \\ & & & \sigma_{44} \end{bmatrix}$$

The REML estimates are indexed as UN (row, column) where row and column indicate the location of estimates in the covariance matrix. (Table 4.9: Type=UNS).

The standard errors for all of the REML estimates in Table 4.9 are asymptotically valid and are obtained from the inverse of the second derivative matrix of the restricted likelihood function. Such estimates of precision are another benefit of a likelihood-based approach, although the asymptotic Wald tests printed at the end of Table 4.9 can be unreliable for small samples.

The variance-covariance structure that best fits the data is selected by comparing among these structures. The Model Fitting Information tables indicated in Table 4.10 present the likelihood- based criteria which are particularly useful in comparing different covariance structure models. As one approach, the Akaike's Information Criteria (AIC) is compared and select the model with the largest value.

Table 4.10: REML Fitting Information

TYPE =CS:

Model Fitting Information	
Description	Value
Observations	528
REML Log Likelihood	263.20
Akaike's Information Criterion	- 522.40
Schwarz's Bayesian Information Criterion	- 516.60
- 2 REML Log Likelihood	- 526.40
Null Model LRT Chi-Square	1.66
Null Model LRT DF	1.0000
Null Model LRT P-Value	0.1981

TYPE =HF:

Model Fitting Information	
Description	Value
Observations	528
REML Log Likelihood	273.0
Akaike's Information Criterion	- 536.0
Schwarz's Bayesian Information Criterion	- 521.6
-2 REML Log Likelihood	- 546.0
Null Model LRT Chi-Square	21.32
Null Model LRT DF	4.00
Null Model LRT P-Value	0.1981

TYPE =AR (1):

Model Fitting Information	
Description	Value
Observations	528
REML Log Likelihood	262.45
Akaike's Information Criterion	-520.90
Schwarz's Bayesian Information Criterion	-515.10
- 2 REML Log Likelihood	-524.90
Null Model LRT Chi-Square	0.18
Null Model LRT DF	1.00
Null Model LRT P-Value	0.6724

TYPE =UNS:

Model Fitting Information	
Description	Value
Observations	528
REML Log Likelihood	279.65
Akaike's Information Criterion	-539.30
Schwarz's Bayesian Information Criterion	-510.50
- 2 REML Log Likelihood	-559.30
Null Model LRT Chi-Square	34.63
Null Model LRT DF	9.00
Null Model LRT P-Value	< 0.0001

AIC favors autoregressive structure followed by the compound symmetry (Table 4.10). Restricted likelihood ratio tests can also be constructed by subtracting the values of - 2 REML Log Likelihood and comparing the result with a tabulated chi-square having degrees of freedom equal to the difference in the number of parameters.

These tests are appropriate for structures which are nested within each other, or in other words, one is a special case of the other. A restricted ratio test between Type H and unrestricted has a calculated chi-square equal to -546.0 - (-559.3) = 13.3. But this value is the Mauchley sphericity test statistic

$\eta_1 \log \omega$ shown in Table 4.3 of Univariate ANOVA. Using 5 (10-5) degrees of freedom, this test favors the Type H structure at $\alpha = 0.01$(p = 0.0221). Compound symmetry is a special case of Type H, and the restricted likelihood ratio test between these two structures has a chi-square value equal to -526.4 - (-546.0) = 19.6 on 3 (5 - 2) degrees of freedom. This test favors compound symmetry.

In univariate analysis of variance section (Section 4.3), it is observed that the data fit the compound symmetry structure since the chi-square calculated for sphericity test is not significant at smaller level of significance. However, one may suspect that the data might have deviations from the compound symmetry structure due to the fact that the chi-square test rejects sphericity assumption at 0.05 level of significance (p = 0.0221).

In the mixed model approach, the AIC value for autoregressive structure is found to be larger as compared to compound symmetry (-522.4 vs -520.90). Thus, by the AIC criterion, one may conclude that the data follows an autoregressive structure.

However, the last three rows of the third sub-table in Table 4.10 are a restricted likelihood ratio tests of the autoregressive covariance model versus the simple structure used in the standard ordinary least squares (σ^2 times an identity matrix). This test indicates that the autoregressive model does not fit better than the simple null model (p=0.6724). Hence, we go back to the compound symmetry structure since it has the next largest AIC value. Compound symmetry structure is also compared with the ordinary least squares simple structure and the test result shows that compound symmetry structure fits better than the simple null model as compared to autoregressive structure (p=0.1981). Hence, the compound symmetry is selected as a best covariance model for the *enset* data among the remaining structures considered.

Using the compound symmetry structure, tests of main effects of variety and time as well as the interaction effects of variety by time will follow. Table 4.11 displays the test of Fixed Effects.

Table 4.11: Tests of Fixed Effects

Source	Num DF	Den DF	F Value	Pr > F
Variety	32	99	16.52	< 0.0001
Time	3	297	549.29	< 0.0001
Variety × Time	96	297	6.33	< 0.0001

As can be seen in Table 4.11, the F values are the same as the ones obtained in the univariate analysis of variance. That means variety, time and variety by time interaction are significant ($p < 0.0001$).

While comparing the methods used in this work, univariate analysis is preferable to its multivariate counterpart in several aspects. It is simpler to fit, it is more powerful, and the results are generally easier to interpret than those of multivariate tests. However, univariate ANOVA method of repeated measures is very restrictive. That is, it requires the assumption of equal variance at all times and pairs of measurements on the same subject to be equally correlated, regardless of the time lag between the measurements, which is not the case in most repeated measures data. When one uses a univariate approach but the requisite statistical assumptions are not met, the significance levels for statistical tests (such as the F values in ANOVA) are no longer accurate, leading to faulty tests of hypothesis.

The advantage of multivariate approach over the univariate is that it has less restrictive assumptions, i.e., MANOVA imposes no restrictive assumptions on the form of the error covariance matrix. In fact it assumes that this matrix is unstructured and sample size is large. But it is less powerful given that the data fulfill type H covariance structure because there are more parameters to estimate and therefore fewer degrees of freedom. The choice between the multivariate approach and univariate repeated measures ANOVA depends on sample size, power and whether statistical assumptions of univariate ANOVA are met.

The advantage of the mixed model over the other models is that the former helps to select appropriate variance-covariance structure and estimate the parameters. The results such as estimated means and relevant tests made would be adjusted for the possible correlation between consecutive time points using the estimated covariance structure. Also the mixed model method is used to analyze data that have missing observations at random.

For the *enset* data used in this analysis, the assumption of repeated measures analysis of variance is fulfilled. Hence, the ANOVA test is valid without adjusting for degrees of freedom. In the multivariate approach, since the sample size which is equal to 528 (i.e., 33 varieties, within each variety there are four replications of *enset* plants each measured 4 times) is large the MANOVA method also gives reliable results. When analyzing the *enset* data using the multivariate methods, no assumption is made about the covariance structure. That is, the covariance form is unrestricted. In the mixed model analysis of the *enset* data, the covariance structure itself is modeled rather than being tested which is the case in univariate ANOVA approach. Then using the selected covariance structure of the data, main and interaction effect tests are performed. This is an advantage of the mixed model since it enables us to know exactly the covariance structure of the *enset* data; hence, the conclusions drawn from the tests based on mixed models are very precise. There are highly significant differences between the factor levels of interest and their interaction using the three models. This happened probably due to the fact that varieties showed extremely high variability among themselves. However, the advantages of the methods used would have been evident had differences between varieties (and their interaction with time) been marginal. In fact there are no missing observations in the *enset* data. But the mixed models still give better results for missing and unbalanced data. Hence it is preferable to use mixed models to analyze the *enset* data.

The analyses results discussed in previous sections using univariate, multivariate and mixed model methods suggest that there are significant main effects for variety and time as well as significant variety by time interaction effects.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Repeated measures analysis was conducted on thirty three varieties of *enset* plants. The data are repeated measurements of pseudostem circumference of each plant measured at four successive time points. Each variety has four replications.

The general objectives were to study the applications of various methods used in repeated measures analysis and the conditions under which each method is applied on repeated measurements of *enset* pseudostem circumference; specifically to test whether mean yield of one *enset* variety varies from the other, to investigate whether time has effect on *enset* yield and to examine whether there is interaction effect between variety and time.

Four different methods were used to analyze the repeated measurements of *enset* plants. These are: 1) Summary statistics; 2) Univariate analysis of variance (split-plot in time ANOVA); 3) Multivariate analysis of Variance (MANOVA) and 4) Mixed model approach.

In summary statistics, the mean of each *enset* variety is provided in a table as a descriptive approach to see how the varieties differ from one another. Moreover, the mean of each *enset* variety is plotted against time on the same graph by connecting the mean of each variety at consecutive time points by straight line. This helps to observe the pattern each variety follows through time as well as the similarity and differences among the varieties. The time at which the varieties attain maximum and minimum pseudostem circumference and the presence or absence of interaction effect of variety with time can also be seen.

In univariate analysis of variance, first the underlying assumptions of ANOVA are checked. Since the data are repeated measurements, the appropriate covariance structure of the within subject factor levels (i.e., measurements taken from the same plant at different times) should be justified in order to apply the repeated measures ANOVA. The normality of the errors, homogeneity of error variance and zero mean of the error term are tested and found to be fulfilled for the *enset* data. Moreover, by sphericity test of the transformed variables using the Mauchley's Criterion, the data satisfy the compound symmetry structure at 0.01 level of significance. Thus, it was possible to apply the repeated measures ANOVA F tests without adjusting for degrees of freedom. From this test, it was concluded that there was significant variety and time main effects as well as significant variety by time interaction effects. But there was no significant mean difference among plants within the same variety.

Another method used to analyze the within-subject effect tests was the multivariate analysis of variance (MANOVA). Unlike, the univariate method, this test is not restrictive because no assumption is made about the covariance structure. Wilk's Lambda statistic was used to test the hypothesis of no time and no time × variety interaction effect and both tests were found to be significant.

Finally, the mixed model was used. To make use of this method, first parameters of the various covariance structures were estimated and the one that best fitted the data was selected by the Akaike's Information criterion (AIC). Using this selected structure, the main effects of variety and time; and the interaction effect of variety × time were tested. The conclusion was that all these tests were significant.

On the other hand, in the literatures reviewed in Section 2.4, Shank and Ertiro (1996) concluded that pseusostem circumference and pseudostem height have a positive linear relationship and high correlation with *enset* yield. Hence, using these authors' results, the conclusions made for *enset* pseudostem circumference can also hold for the *enset* yield.

Therefore, based on Shank and Ertiro (1996) and the results of the repeated measures analyses considered in this study, we concluded that the different *enset* varieties yield significantly different yields. That is, some varieties give higher yields while others give smaller and medium yields. Hence, agronomists and breeders should select among the varieties which give optimum yield. They should also duplicate these varieties and distribute to the farmers. Time has also significant effect on *enset* yields. This implies that if *enset* is harvested at different times, its yield differs. It is clear that *enset* that is harvested at maturity gives better yields than that at younger ages. Moreover, there is significant variety by time interaction. That means, even though yield increases as the plant approaches to its maturity, the time at which the plant matures varies from one variety to another and the rate of increase in yield also varies from variety to variety. Therefore, farmers and agricultural researchers should know whether a variety reaches to maturity before harvesting the plant in order to get better yields. Harvesting all varieties at one and the same time may result in minimal yield because according to the results of the study, the varieties mature at different times.

Finally, from the repeated measures analyses used in this study, the following are recommended:

- It is found that the mixed model is the best method to analyze the repeated measurements of *enset* data since it enables to model the covariance structure of the data before analysis, adjust means and improve tests on the main and interaction effects based on the selected structure. It would also be helpful in a situation where there are missing values in the data. Hence, it is recommendable to use mixed models to analyze repeatedly measured data in order to get better results.

- Using the findings in this study as preliminary results, post-hoc analyses like multiple comparison tests can be done to select high yielding variety among those considered in the analysis.

- From the fact that variety and time have significant interaction effects, it can be further studied at what time which variety yields better results and it is also possible to model the interaction type.

- This study can be duplicated to other research centers to identify the variety which gives stable yields at all locations and which variety gives best results under which environment and disseminating those varieties throughout the *enset* growing areas where they perform better.

REFERENCES

Agren, G. and R. Gibsen (1968). Food Composition Table for use in Ethiopia. *Nutrition Unit Report No.* 16.

Anderson, T.W. (1958). An Introduction to Multivariate Statistical Analysis. *New York: John Wiley & Sons, Inc.*

Box, G.E.P (1954). Some Theorems on Quadratic Forms Applied in the Study of Analysis of Variance Problems: II. Effects of Inequality of Variance and of Correlation between Errors in the Two-Way Classification. *Annals of Mathematical Statistics,* 25, 484-498.

Bozdogan, H. (1987). Model Selection and Akaike's Information Criteria (AIC): The General Theory and Its Analytical Extensions. *Psychometrika* 52, 345-370.

Central Statistics Authority (1995). Agricultural Sample Survey 1990/91 Meher Season. *Report on Agricultural Practices, Statistical Bull.* 130:31-34.

Cheesman, E.E (1947). Classification of the Bananas. I. the Genus Ensete Horan. *Kew Bull.* 2: 97-117.

Davidson, M.L. (1972). Univariate versus Multivariate Test in Repeated Measures Experiments. *Psychological Bulletin* 77, 446-452.

Diggle, P.J. (1988). An Approach to the Analysis of Repeated Measurements. *Biometrics,* 44, 959-971.

Frison, L. and Pocock, S.J (1992). Repeated Measures in Clinical Trials: Analysis Using Mean Summary Statistics and Its Implication for Design," *Statist. Med.,* 11, 685-1704.

Greenhouse, S.W, and Geisser, S. (1959). On Methods in the Analysis of Profile Data. *Psychometrika,* 24, 95-111.

Gorgulu, O. and Sahiler, S. (2001). Repeated Measures Analysis and Some Experimental Design Considerations in Animal Science. *Mustafa Kemal University, Agriculture Faculty, Antakya/Hatay.*

Hearne, E. M. III Clark, G. M. Hatch, J. P. (1983). A Test for Serial Correlation in Univariate Repeated Measures Analysis. *Biometrics,* Vol. 39, No. 1, pp 237-243

Henderson, C.R. (1984). Applications of Linear Models in Animal Breeding. *University of Guelph, Guelph, Ontario.*

Hetzog, C. and Rovine, M. (1985). Repeated Measures Analysis of Variance in Developmental Research: Selected Issues. *Pennsylvania State University*

Huynh H. and Feldt, L.S. (1976). Estimation of the Box Correction for Degrees of Freedom from Sample Data in Randomized Block and Split-Plot Designs. *Journal of Educational Statistics, Volume1, No. 1,* pp 69-82.

Huynh, H. (1978). Some Approximate Tests for Repeated Measurement Designs. *Psychometrika,* 43, 161-175.

Huynh, H., and Feldt, L.S. (1970). Conditions under Which Mean Square Ratios in Repeated Measures Designs Have Exact F Distributions. *Journal of American Statistical Association,* 65, 1582-1589.

Huynh, H. and Mandeville, G.K. (1979). Validity Conditions in Repeated Measures Designs. *Psychological Bulletin*, 86, 964-973.

Jennrich, R.I. and Schluchter, M.D. (1986). Unbalanced Repeated Measures Models with Structured Covariance Matrices. *Biometrics,* 42, 805-820.

Johnson, R. A and Wichern, D.W. (2002). Applied Multivariate Statistical Analysis. *Fifth Edition, Upper Saddle River N.J Prentice Hall.*

Jones, M.C. and Rice, J.A (1992).Displaying the Important Features of a Large Collection of Similar Curves. *Am. Statistn,* 46, 140-145.

Krik, R.E (1982). Experimental Design (2^{nd} ed.). *Belmont, CA: Brooks-Cole.*

Lindsey, J.K. (1993). Models for Repeated Measurements. *Oxford: Clarendon Press.*

Littel, R.C, Milliken, G.A., Stroup, W.W., Wolfinger, R.D. and Schabenberger, O. (2006). SAS for Mixed Models, (2^{nd} ed.). *SAS Institute Inc., Cary, North Carolina, USA.*

Mauchley, J.W (1940). Significant Test for Sphericity of a Normal n-Variate Distribution. *The Annals of Mathematical Statistics,* 11, 204-209.

Mccall, R.B. and Appelbaum, M.I. (1973). Bias in the Analysis of Repeated Measures Designs: Some Alternative Approaches. *Child Development,* 44, 401-415.

Morrison, D.F. (1976). Multivariate Statistical Methods (2^{nd} ed.). *New York: McGraw-Hill.*

Nagarsenker, B.N., and Pillai, K.C.S (1973). The Distribution of the Sphericity Test Criterion. *Journal of Multivariate Analysis, 3, 226-235.*

Purseglove, J. W (1972). Musaceae. P.343-384. In: Tropical Crops. Monocotyledons 2. *Longman Group Limited, London.*

Rogan, J.C., Keselman, H.J., and Mendoza, J.L. (1979). Analysis of Repeated Measurements. *British Journal of Mathematical and Statistical Psychology,* 32, 269-286.

Rubin, D.B (1976). Inference and Missing Data. *Biometrika, 63, 581-592.*

SAS Institute Inc. (1992). SAS/STAT User's Guide: Version 6, Fourth Edition, Volume 2, Cary NC. *SAS Institute Inc.*

Scheifly, V.M., and Schmidt, W.H. (1978). Analyses of Repeated Measures Data: A Simulation Study. *Multivariate Behavioral Research,* 13, 347-362.

Searle, S.R (1971). Linear Models. *New York: John Wiley & Sons, Inc.*

Shank, R. and Ertiro, C. (1996). Enset Crop Assessment. *United Nations World Food Programme.*

Smeds, H. (1955). The Ensete Planting Culture of Eastern Sidamo, Ethiopia. *Acta Geography Helsingfors* 13(4):4.

Spring, A. (1996). *Enset* Farming System in Southern Region, Ethiopia. Report on a Rapid Rural Appraisal in Gurage, Hadiya and Sidama Zones. *Enset Needs Assessment Project Phase I, Awasa, Ethiopia*

Taye Bezuneh, Asrat Felleke, and Regassa Bayie (1967). The Cultivation of the Genus Ensete in Ethiopia. *Soil and Crop Science Society, Florida* 27: 133-141.

Vavilov, N.I (1957). World Resources of Cereals, Grains Leguminous Crops and Flax, their Utilization in Plant Breeding. *Moscow.*

Westphal, E. (1975). Agricultural Systems in Ethiopia. *Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands*

Winer, B.J. (1971). Statistical Principles in Experimental Design, 2nd ed.," *New York: McGraw-Hill.*

Wolfinger, R.D. (1993). Covariance Structure Selection in General Mixed Models. *Communications in Statistics, Simulation and Computation,* 22(4), 1079-1106.

APPENDIX A

The statistical analyses presented in this thesis were analyzed using SAS software (SAS, 1992). The SAS program statements are presented below. To mention some of the SAS procedures, PROC GLM was used to analyze the Univariate analysis of variance (ANOVA) and Multivariate analysis of variance (MANOVA). The Greenhouse-Geisser (G-G) and Huynh–Feldt (H-F) estimates were also outputs of the PROC GLM. Pseudostem circumference means against time were plotted using PROC PLOT. In the Mixed Model Approach, PROC MIXED was used to get the various covariance structures from which we selected the structure which fits best our data. The Akaike's Information Criterion (AIC) and the Likelihood Ratio Estimates were also obtained from the PROC MIXED.

a) SAS Codes for ANOVA and MANOVA

```
Proc GLM Data = Enset;
  Class variety plant time;
  Model stemcir = variety plant (variety) time variety*time;
  Test h=variety e=plant (variety);
  Lsmeans variety;
  Output out=bell rstudent=res1 p=pred1;
  Run;
Proc glm data=new;
  Class variety;
  Model stemcir1-stemcir4=variety/nouni;
  Repeated time 4 polynomial/printe summary;
  Lsmeans variety;
Proc glm data=new;
  Class variety stemcir1 stemcir2 stemcir3 stemcir4;
  Model stemcir1 stemcir2 stemcir3 stemcir4=variety;
  Repeated time 4;
  Lsmeans variety /out=means;
  Quit;
Run;
Proc sort data =repeated_measures;
  By variety plant;
Data new (keep=stemcir1-stemcir4 variety);
  Array stemcirstemcir (4) stemcir1-stemcir4;
  Do time=1 to 4;
     Set repeated_measures;
     By variety plant;
     Stemcirstemcir (time)=stemcir;
     If last. Plant then return;
    End;
 Run;
Proc print;
Run;
```

b) SAS Codes for Residual Analysis

```
Proc print data=bell;
Run;
Proc univariate plot normal data=bell;
Var res1;
Run;
Proc plot data=bell;
Plot res1*pred1;
Run;
```

c) SAS Codes for plotting pseudostem circumference against Time

```
Goptions reset=all;
Symbol1 c=blue v=a h=1 i=j;
Symbol2 c=red   v=b   h=1 i=j;
Symbol3 c=green v=c h=1 i=j;
Symbol4 c=red   v=d h=1 i=j;
Symbol5 c=black v=e h=1 i=j;
Symbol6 c=yellow v=f h=1 i=j;
Symbol7 c=purple v=g h=1 i=j;
Symbol8 c=magenta v=h h=1 i=j;
Symbol9 c=gray v=i h=1 i=j;
Symbol10 c=brown v=j h=1 i=j;
Symbol11 c=blue v=k h=1 i=j;
Symbol12 c=blue v=l h=1 i=j;
Symbol13 c=blue v=m h=1 i=j;
Symbol14 c=blue v=n h=1 i=j;
Symbol15 c=blue v=o h=1 i=j;
Symbol16 c=blue v=p h=1 i=j;
Symbol17 c=red   v=q   h=1 i=j;
Symbol18 c=green v=r h=1 i=j;
Symbol19 c=red   v=s h=1 i=j;
Symbol20 c=black v=t h=1 i=j;
Symbol21 c=blue v=u h=1 i=j;
Symbol22 c=blue v=v h=1 i=j;
Symbol23 c=blue v=w h=1 i=j;
Symbol24 c=blue v=x h=1 i=j;
Symbol25 c=blue v=y h=1 i=j;
Symbol26 c=blue v=z h=1 i=j;
Symbol27 c=blue v=1 h=1 i=j;
Symbol28 c=blue v=2 h=1 i=j;
Symbol29 c=blue v=3 h=1 i=j;
Symbol30 c=blue v=4 h=1 i=j;
Symbol31 c=blue v=5 h=1 i=j;
Symbol32 c=blue v=6 h=1 i=j;
Symbol33 c=blue v=7 h=1 i=j;
Axis1 order= (0 to 1.5 by .1) label = (a= 10 'means');
Axis2 label = ('time') value= ('1' '2' '3' '4');
Proc Gplot data=means;
Plot lsmean*_name_=variety/vaxis=axis1 haxis=axis2;
Run;
Quit;
```

d) SAS Codes for Mixed Model Analysis

```
Proc Mixed Data = Repeated_Measures_Mixed covtest;
 Class variety plant time;
 Model stemcir = variety time Variety*time;
 Repeated time/ Sub=Plant (Variety) Type=CS r rcorr;
 Run;
Proc Mixed Data = Repeated_Measures_Mixed covtest;
 Class variety plant time;
 Model stemcir = variety time Variety*time;
 Repeated time/Sub=Plant (Variety) Type=hf r rcorr;
 Run;
Proc Mixed Data = Repeated_Measures_Mixed covtest;
 Class variety plant time;
 Model stemcir = variety time Variety*time;
 Repeated time/Sub=Plant (Variety) Type=ar (1) r rcorr;
 Run;
Proc Mixed Data = Repeated_Measures_Mixed covtest;
 Class variety plant time;
 Model stemcir = variety time Variety*time;
 Repeated time/Sub=Plant (Variety) Type=arh (1) r rcorr;
 Run;
Proc Mixed Data = Repeated_Measures_Mixed covtest;
 Class variety plant time;
 Model stemcir = variety time Variety*time;
 Repeated time/Sub=Plant (Variety) Type=UN r rcorr;
 Run;
```

APPENDIX B

In order to check whether our data fulfill the assumptions for Repeated Measures ANOVA, we conduct the Residual Analyses and get the following SAS outputs:

a) Residual Analysis for Zero Error Variance

```
                         The UNIVARIATE Procedure
                            Variable:  res1

                                Moments

    N                            528    Sum Weights                528
    Mean                  0.00002581    Sum Observations    0.01362925
    Std Deviation         1.00437754    Variance            1.00877425
    Skewness              0.01311193    Kurtosis            0.05098931
    Uncorrected SS        531.624031    Corrected SS         531.62403
    Coeff Variation       3890980.42    Std Error Mean      0.04370992


                      Basic Statistical Measures

            Location                    Variability

        Mean     0.00003     Std Deviation            1.00438
        Median  -0.04753     Variance                 1.00877
        Mode    -0.51006     Range                    6.65838
                             Interquartile Range      1.28845

 NOTE: The mode displayed is the smallest of 14 modes with a count of 2.


                      Tests for Location: Mu0=0

          Test           -Statistic-    -----p Value------

          Student's t    t  0.000591    Pr > |t|    0.9995
          Sign           M       -10    Pr >= |M|   0.4083
          Signed Rank    S      -789    Pr >= |S|   0.8223
```

b) Residual Analysis for Normality of Errors

```
                          Tests for Normality

       Test                  --Statistic---    -----p Value------

       Shapiro-Wilk          W      0.99674    Pr < W      0.3666
       Kolmogorov-Smirnov    D     0.037299    Pr > D      0.0743
       Cramer-von Mises      W-Sq  0.101405    Pr > W-Sq   0.1096
       Anderson-Darling      A-Sq  0.595609    Pr > A-Sq   0.1247
```

```
                      Quantiles (Definition 5)
                      Quantile        Estimate
                      100% Max       2.9547435
                      99%            2.2041101
                      95%            1.7194962
                      90%            1.3696408
                      75% Q3         0.6398964


                     The UNIVARIATE Procedure
                          Variable:  res1

                      Quantiles (Definition 5)

                      Quantile        Estimate

                      50% Median    -0.0475272
                      25% Q1        -0.6485559
                      10%           -1.2476109
                      5%            -1.6230932
                      1%            -2.2484063
                      0% Min        -3.7036370


                        Extreme Observations

            ------Lowest-----         -----Highest-----

               Value      Obs            Value      Obs

            -3.70364       55          2.23954       63
            -2.64916      272          2.27500      208
            -2.61338      370          2.43494      138
            -2.46166      400          2.66707      270
            -2.25727       57          2.95474       56

                  Histogram                      #             Boxplot
 2.75+*                                          2                0
     .****                                      11                |
     .**********                                29                |
     .****************                          46                |
     .*************************                 73             +-----+
     .*******************************           93             |  +  |
     .************************************     106             *-----*
     .*******************************           93             +-----+
     .**************                            41                |
     .*******                                   21                |
     .****                                      10                |
     .*                                          2                0
     .
-3.75+*                                          1                0
      ----+----+----+----+----+----+----+-
      * may represent up to 3 counts
```

```
                     The SAS System
              The UNIVARIATE Procedure
                  Variable:  res1

                   Normal Probability Plot
 2.75+                                                 *
     |                                            ******
     |                                      ******
     |                                  *****
     |                             ******
     |                        ******
     |                   ******
     |             *******
     |         *****+
     |     *****
     |******
     |*
     |
-3.75+*
      +----+----+----+----+----+----+----+----+----+----+
         -2        -1         0        +1        +2
```

c) Residual Analysis for Homogeneity of Error Variance