

**ANALYSIS OF THE LETTUCE DATA FROM THE VARIABLE PRESSURE GROWTH  
CHAMBER AT NASA-JOHNSON SPACE CENTER:  
A THREE-STAGE NESTED DESIGN MODEL**

**Final Report**

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## ABSTRACT

A model of three-stage nested experimental design was applied to analyze the lettuce data obtained from the variable pressure growth chamber test bed at NASA-Johnson Space Center. From the results of an application of the analysis of variance and covariance on the data set, it was noted that all of the (uncontrollable) factors, Side, Zone, Height and (controllable) PAR (photosynthetically active radiation), had nonhomogeneous effects on the dry weight of the edible biomass of lettuce per pot. Incidentally, the variations accountable to the (uncontrollable) factorial heterogeneities are merely 9% and 17% of the total variation for both the first and second crop test, respectively. After adjusting for the PAR as a covariate in the no-intercept model, the accountable variations to all the four factors are 94% and 92% for the first and the second crop test, respectively. With the use of a no-intercept simple linear regression model, the accountable variations to the factor PAR are 92% and 90% for the first and the second crop test, respectively. Evidently, the (controllable) factor PAR is the dominating one.

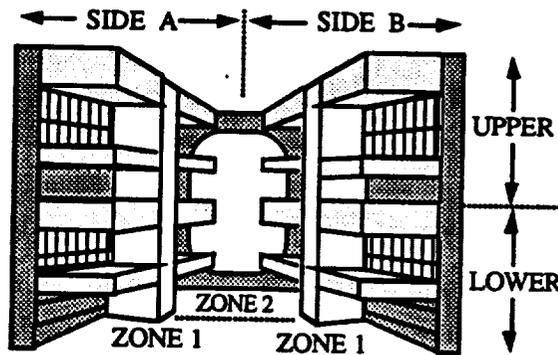
## 1. INTRODUCTION

The aim of this report is to apply a three-stage nested experimental design in modeling the lettuce data generated from the variable pressure growth chamber (VPGC) test bed at NASA's Johnson Space Center (Tri, et al 1991). The purpose of the research is that for long duration space missions such as a Lunar or Martian outpost technologies will be needed to revitalize atmospheric constituents, to process wastes, to regenerate water, and to produce food for human consumption under the premises of minimizing dependency on resupply from earth and attaining self-sufficiency. NASA's Controlled Ecological Life Support Systems Program (CELSS) was studying the use of biological processes for integration into regenerative life support systems. Higher plants could be used as an integral part of these life support systems, because they remove carbon dioxide and produce oxygen through photosynthesis, purify water through transpiration, and produce food (Schwartzkopf 1992).

The data set used in this report was the same as that of Barta, et al (1992). As a result of the specific engineering design of the growth chamber test bed, it was noted that the factor Zone (representing four independent nutrient solution irrigation systems) was nested within the factor Side (representing two atmospheric conditioning systems), while the factor Height (representing the upper and lower growing area) was nested within the factor Zone. The tests were conducted under ambient atmospheric pressures in the VPGC, a vacuum chamber outfitted for plant growth. The VPGC encloses a total of 10.6 m<sup>2</sup> of area for crop growth, split into eight individual growing areas (Figure 1.1). Two atmospheric conditioning systems are present, one on each chamber side supporting four individual growing areas. Four independent nutrient solution irrigation systems are present. Each irrigation system, or zone, supports a pair of growing areas (one upper and one lower growing areas). A complete description of the chamber and its plant support systems is given in Tri, et al (1991). Two crop tests were replicated. The environmental conditions and cultural practices used during both crop tests are presented in Table 1.1. For both tests, each growing area was outfitted with 60 pots, for a total of 480 pots within the chamber. Each pot was filled with approximately 250 ml of calcined clay.

**Table 1.1.** Environmental Conditions and Cultural Practices Used During the First and Second Crop Test.

Parameter	Units	Crop Test	
		First	Second
Average Air Temperature	°C	22.8	23.1
Average Relative Humidity	%	73	72
Carbon Dioxide Level	$\mu\text{L L}^{-1}$	1000	1000
Average Photosynthetic Photon Flux (PPF)	$\mu\text{mol m}^{-2} \text{s}^{-1}$	365	346
Irrigation Frequency	Events day <sup>-1</sup>		
Week 1		1	1
Week 2		2	1
Weeks 3 & 4		3	3
Irrigation Amount	ml pot <sup>-1</sup> event <sup>-1</sup>	37	30



**Figure 1.1.** Interior Layout of the Variable Pressure Growth Chamber (VPGC).

Two seeds of lettuce were planted within each pot. The pots were irrigated with a modified half-strength Hoagland's nutrient solution. The plants were harvested 30 days after seeding. Here the conditions for each crop were nominally set to be the same for all growing areas.

## 2. STATISTICAL MODELING

A three-stage nested design model without/with the photosynthetically active radiation (PAR) as a covariate and/or with no-intercept term were employed in fitting the collected lettuce data as follows:

$$y_{ijkl} = \mu + \alpha_i + \beta_{(i)j} + \gamma_{(ij)k} + \varepsilon_{(ijk)l}, \quad i = A, B, j = 1, 2, k = H, L, l = 1, \dots, 60; \quad (2.1)$$

$$y_{ijkl} = \mu + \alpha_i + \beta_{(i)j} + \gamma_{(ij)k} + \theta x_{ijkl} + \varepsilon_{(ijk)l}, \quad i = A, B, j = 1, 2, k = H, L, l = 1, \dots, 60; \quad (2.2)$$

and

$$y_{ijkl} = \alpha_i + \beta_{(i)j} + \gamma_{(ij)k} + \theta x_{ijkl} + \varepsilon_{(ijk)l}, \quad i = A, B, j = 1, 2, k = H, L, l = 1, \dots, 60; \quad (2.3)$$

where :

$x_{ijkl}$  - intensity of photosynthetically active radiation (PAR) received at the  $l$ -th plant in the  $k$ -th height within the  $j$ -th zone and the  $i$ -th side,

$y_{ijkl}$  - dry weight (DW) of edible biomass of the  $l$ -th lettuce in the  $k$ -th height within the  $j$ -th zone and the  $i$ -th side,

$\mu$  - mean biomass of all plants in the crop,

$\alpha_i$  - differential effect attributed to the  $i$ -th side ,

$\beta_{(i)j}$  - differential effect attributed to the  $j$ -th zone within the  $i$ -th side,

$\gamma_{(ij)k}$  - differential effect attributed to the  $k$ -th height within the  $i$ -th side and the  $j$ -th zone,

$\theta$  - regression coefficient of  $x_{ijkl}$ ,

$\varepsilon_{(ijk)l}$  - error term assuming to have a normal probability distribution with a mean zero and unknown constant variance  $\sigma^2 > 0$  representing the variation of biomass from plant to plant within each growing area.

Since the effect of the factors are fixed, we assume that the following constraints hold for Eqs. (2.1-2):

$$\sum_i \alpha_i = 0, \quad (2.4a)$$

$$\sum_j \beta_{(ij)} = 0, \quad (2.4b)$$

$$\sum_k \gamma_{(ij)k} = 0. \quad (2.4c)$$

Note that there are no interaction terms among the three factors in Eq. (2.1-3), because it can be shown (Montgomery 1992) that there is no need to include the interaction term in the model of multi-stage nested experimental design.

Table 2.1. Expected Mean Squares for the Three-Stage Nested Design Model of Eq. (2.1).

E(MS)	Side, Zone, Height: fixed
$E(MS_{side})$	$\sigma^2 + 240 \sum_i \alpha_i^2$
$E(MS_{(side)zone})$	$\sigma^2 + 120 \sum_i \sum_j \beta_{(ij)}^2$
$E(MS_{(side,zone)height})$	$\sigma^2 + 60 \sum_i \sum_j \sum_k \gamma_{(ij)k}^2$
$E(MS_{error})$	$\sigma^2$

Table 2.2. Analysis of Variance Table for the Three-Stage Nested Design Model of Eq. (2.1).

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
Side	$\sum_i y_{i..}^2 / 240 - y_{...}^2 / 480$	1	$MS_s$
Zone within side	$\sum_i \sum_j y_{ij.}^2 / 120 - \sum_i y_{i..}^2 / 240$	2	$MS_{(s)z}$
Height within zone, side	$\sum_i \sum_j \sum_k y_{ijk}^2 / 60 - \sum_i \sum_j y_{ij.}^2 / 120$	4	$MS_{(s,z)h}$
Error	$\sum_i \sum_j \sum_k \sum_l y_{ijkl}^2 - \sum_i \sum_j \sum_k y_{ijk}^2 / 60$	472	$MS_e$
Total	$\sum_i \sum_j \sum_k \sum_l y_{ijkl}^2 - y_{...}^2 / 480$	479	

where  $y_{....}$ ,  $y_{i...}$ ,  $y_{ij..}$ , and  $y_{ijk.}$  are defined, respectively, as follows:

$$y_{....} = \sum_i \sum_j \sum_k \sum_l y_{ijkl},$$

$$y_{i...} = \sum_j \sum_k \sum_l y_{ijkl},$$

$$y_{ij..} = \sum_k \sum_l y_{ijkl},$$

$$y_{ijk.} = \sum_l y_{ijkl}.$$

The expected mean squares for Eq. (2.1) is given in Table 2.1. Since the effect of the factors Side, Zone and Height are regarded as fixed, it is noted from Table 2.1 that the null hypotheses  $H_0: \alpha_i = 0$ ,  $H_0: \beta_{(ij)} = 0$ , and  $H_0: \gamma_{(ij)k} = 0$  can be tested by  $MS_{side}/MS_{error}$ ,  $MS_{(side)zone}/MS_{error}$ , and  $MS_{(side,zone)height}/MS_{error}$ , respectively. The test procedure is summarized in an analysis of variance table as shown in Table 2.2.

### 3. RESULTS AND ANALYSIS

All computations were performed on the Macintosh personal computer through the use of the MGLH procedure in SYSTAT (Wilkinson 1987). The analysis of variance and covariance with/without the intercept term for the first and the second crop test are summarized, respectively, in Tables 3.1-3 and 3.4-6. Numerical results summarized in Tables 3.1-6 were obtained by fitting Eqs. (2.1-3) to new data sets after a deletion of those data points which were identified as outliers and having zero dry weight in the first fitting of Eq. (2.1) to the original data set. From Table 3.1, all of the three factors, Side, Zone, and Height, had differential effects on the dry weight of the edible biomass of lettuce at the significance level of less than 1%. From Tables 3.2-3, it was noticed that after adjusting for the influence of the covariate PAR, the effects of Side, Zone, and Height are still significant. Yet the factor PAR is clearly the dominating one. Table 3.7 indicates that the lettuce plants growing, respectively, in Side B, Zone 1, and 'low' growing area had greater dry weights, on the average, than in Side A, Zone 2, and 'high' growing area.

**Table 3.1.** The Analysis of Variance of Eq. (2.1) for the First Crop Test.

Model	Source	Sum of Squares	d.f.	Mean-Square	F-ratio	Pr. > F
3-Stage Nested Design without covariate	Side	16.55	1	16.55	16.55	0.000
	Zone/Side	12.18	2	6.09	5.16	0.004
	Ht/Zone/Side	19.65	4	4.91	4.16	0.002
	Error	504.10	429	$\hat{\sigma}^2 =$ 1.18		
$R^2 = 0.09$						

**Table 3.2.** The Analysis of Covariance of Eq. (2.2) for the First Crop Test.

Model	Source	Sum of Squares	d.f.	Mean-Square	F-ratio	Pr. > F
3-Stage Nested Design with covariate	Side	33.66	1	33.66	34.44	0.000
	Zone/Side	9.53	2	4.77	4.88	0.008
	Ht/Zone/Side	16.68	4	4.17	4.27	0.005
	PAR	85.79	1	85.79	87.54	0.000
	Error	418.31	428	$\hat{\sigma}^2 =$ 0.98		
$R^2 = 0.24$						

Similar results hold for the second crop test as shown in Tables 3.4-6 and 3.8. The dry weight of edible biomass of lettuce in the upper growing area was lighter, on the average, than that of the lower growing area. This may have resulted from a less delivery of nutrient solution to the upper growing area as compared to the lower growing area. The average air temperature and relative humidity for Side A and B over the 30 day crop test were 22.1°C and 23.5°C and 80% and 66.5%, respectively. The warmer conditions present on Side B

**Table 3.3.** The Analysis of Covariance of Eq. (2.3) for the First Crop Test.

Model	Source	Sum of Squares	d.f.	Mean-Square	F-ratio	Pr. > F
3-Stage Nested Design with covariate and no-intercept	Side	40.59	1	40.59	41.08	0.000
	Zone/Side	9.78	2	4.89	4.95	0.005
	Ht/Zone/Side	20.48	4	5.12	5.18	0.000
	PAR	6032.50	1	6032.50	6105.77	0.000
	Error	423.92	429	$\hat{\sigma}^2 = 0.99$		
$R^2 = 0.94$						

**Table 3.4.** The Analysis of Variance of Eq. (2.1) for the Second Crop Test.

Model	Source	Sum of Squares	d.f.	Mean-Square	F-ratio	Pr. > F
3-Stage Nested Design without covariate	Side	20.07	1	20.07	24.18	0.000
	Zone/Side	31.74	2	15.87	19.12	0.000
	Ht/Zone/Side	24.26	4	6.06	7.30	0.000
	Error	382.43	463	$\hat{\sigma}^2 = 0.83$		
	$R^2 = 0.17$					

may have increased the dry weight of edible biomass of lettuce. From Tables 3.3 and 3.6, it was noted that the variation accountable to all of the four factors for Eq. (2.3) were 94% and 92% (the value of  $R^2$ ), which were much higher than the corresponding one for Eq. (2.2), in the total variation of the dry weight of edible biomass of lettuce for the first and

**Table 3.5.** The Analysis of Covariance of Eq. (2.2) for the Second Crop Test.

Model	Source	Sum of Squares	d.f.	Mean-Square	F-ratio	Pr. > F
3-Stage Nested Design with covariate	Side	25.14	1	25.14	39.28	0.000
	Zone/Side	21.48	2	10.74	16.78	0.000
	Ht/Zone/Side	23.07	4	5.77	9.02	0.000
	PAR	86.66	1	86.66	135.41	0.000
	Error	295.77	462	$\hat{\sigma}^2 =$ 0.64		
$R^2 = 0.37$						

**Table 3.6.** The Analysis of Covariance of Eq. (2.3) for the Second Crop Test.

Model	Source	Sum of Squares	d.f.	Mean-Square	F-ratio	Pr. > F
3-Stage Nested Design with covariate and no-intercept	Side	25.00	1	25.00	39.12	0.000
	Zone/Side	22.13	2	11.07	17.32	0.000
	Ht/Zone/Side	23.09	4	5.77	9.03	0.000
	PAR	3222.96	1	3222.96	5042.77	0.000
	Error	295.92	463	$\hat{\sigma}^2 =$ 0.64		
$R^2 = 0.92$						

second crop test, respectively. It indicates that a no-intercept model of Eq. (2.3) fits the lettuce data much better than the model of Eq. (2.2) as far as the explainable variation due to the inclusion of covariate PAR in the model is concerned. Also, we note that although the error sum of squares for the second crop test is smaller than that of the first crop test, the dry weight of the edible biomass of lettuce for the first crop test is heavier than that for

**Table 3.7. The Summary Statistics for the First Crop Test.**

Factor	Level	No. of cases	Minimum	Maximum	Mean	s.d.
Side	A	222	0.508	6.3	3.495	1.011
	B	215	0.0	7.5	3.895	1.204
Zone	1	219	1.10	7.5	3.854	1.140
	2	218	0.0	7.4	3.529	1.173
Height	H	217	0.0	7.4	3.477	0.988
	L	220	0.508	7.5	3.904	1.213

**Table 3.8. The Summary Statistics for the Second Crop Test.**

Factor	Level	No. of cases	Minimum	Maximum	Mean	s.d.
Side	A	239	0.0	5.4	2.372	0.917
	B	232	0.10	5.4	2.783	1.016
Zone	1	235	0.7	5.4	2.830	0.927
	2	236	0.0	5.2	2.321	0.982
Height	H	238	0.1	4.2	2.355	0.824
	L	233	0.0	5.4	2.799	1.087

the second crop test. This is probably attributed to the less irrigation frequency in Week 2 for the second crop test and less delivered nutrient solution per irrigation event (Table 1.1). Incidentally, a checking for the validity of normality and independence assumption were carried out for all the model fitting exercises by plotting the residuals versus the predicted value of the dry weight of edible biomass of lettuce and a normal probability plot of residuals, respectively. The model assumptions of independence and normality were judged to be satisfactory for all the fitted models by visualization of the plots. The Pearson's correlation coefficient between DW and PAR for the first and the second crop test are 0.37 and 0.45 which were shown to be significantly different from zero. In fact,

after examining the plots of residuals for the validation of independence and normality assumptions, a simple no-intercept linear regression model given by

$$\hat{D}W = 0.01*PAR \text{ (or } = 0.008*PAR) \quad (3.1)$$

was determined to be adequate with  $R^2 = 0.92$  (or 0.90) and  $\hat{\sigma}^2 = 1.13$  (or 0.78) in describing a strong linear relationship between the response variable DW and the predictor variable PAR for the first (or second) crop test. As compared with a partially nested design model used in Barta (1992), the fully nested design models of Eqs. (2.1-3) are preferred since the interaction between the factors Side and Height was shown to be not significant as a result of hypothesis testing.

#### 4. CONCLUSION

Based upon the present analysis of the lettuce data, it is noted that the effects of two atmospheric conditioning systems, four independent nutrient solution irrigation systems, and two growing (high or low) areas on the plant biomass production are not homogeneous. This implies that the growth chamber environment is not spatially uniform. This phenomenon of nonuniformity even in controlled growth chambers was also observed in Lee-Rawlings (1982). Fortunately, the variation accountable to the three (uncontrollable) factors, Side, Zone, and Height, in the total variation of the dry edible biomass of lettuce are no more than 2% for either the first or the second crop test after adjusting for the (controllable) factor PAR (photosynthetically active radiation) as a covariate in the no-intercept model, the accountable variation for all the (uncontrollable and controllable) factors is more than 92% for both the first and the second crop test. With the use of a no-intercept simple linear regression model, the accountable variation for the factor PAR is more than 90% for both the first and the second crop test. Evidently, the (controllable) factor PAR is the dominating one. Further studies seem warranted to find the best combination of factor levels for the controllable factors which might provide the maximum yield of the dry edible biomass of lettuce.

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