

# THE USE OF CONTRASTS IN MULTIVARIATE NONLINEAR MIXED MODELS TO COMPARE TREATMENTS IN LONGITUDINAL FACTORIAL EXPERIMENTS

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- **ABSTRACT:** The purpose of this study was to establish contrasts in multivariate nonlinear mixed models to verify the effects of treatments in experiments with longitudinal data and multiple responses. The evaluated nonlinear functions were the three parameters curves logistic, Gompertz and von Bertalanffy. The random variables were added to the fixed parameters, asymptote  $\alpha$ , abscissa of the inflection point  $\beta$ , and parameter  $\gamma$ . The best adjusted model was expanded with covariates, which establish orthogonal contrasts, in order to verify main effects and interactions in factorial experiments. The methodology was applied to analyse data of an experiment with citrus, in which case the logistic bivariate mixed effects model was the best fit. The chosen model allowed comparisons between treatments in a global context of more than one dependent variable and throughout the measurement period.
- **KEYWORDS:** Nonlinear regression; asymptotes models; dummy variables; citrus data.

## 1 Introduction

In longitudinal experiments with multiple responses, the use of multivariate nonlinear mixed models may be advantageous in comparison with the univariate model. Among other advantages, the joint analysis can evaluate the relation between the treatments and all the responses simultaneously (VERBEKE *et al.*, 2014). The superiority of the multivariate model, compared with the univariate, was

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related in several articles as Strathe et al., 2011, Kuramoto et al., 2013. In addition, when there are many individuals, each one with growth measurements over time, the nonlinear mixed effects models are more appropriate than fixed effects because it is possible to evaluate growth at both the individual unit level, as well as the population level (HARRING and BLOZIS, 2014; LI and JIANG, 2013; REGADAS FILHO *et al.*, 2014). Nonlinear models are widely employed not only to quantify growth, but also to make comparisons between the parameters of the model, to verify the influence of treatment groups in planned experiments (REGADAS FILHO *et al.*, 2014; STRATHE *et al.*, 2010; KARADAVUT *et al.*, 2017).

The purpose of this study was to establish contrasts in multivariate nonlinear mixed models to verify the effects of treatments in longitudinal factorial experiments with multiple responses. The logistic, Gompertz and von Bertalanffy functions were employed, and the models were implemented with dummy-variables to make the comparisons between treatments. As an example of application of the methodology we analysed production and trunk circumference data of sweet orange trees, with five cups budded on five rootstocks, over six years.

## 2 Materials and methods

The analyses were developed in three steps. In step 1 fixed univariate models are adjusted with the objective of verifying the adjustment of the logistic, Gompertz and von Bertalanffy functions to the experimental data. The observation for the  $i$ th individual,  $i = 1, \dots, N$  at time  $x_j$ ,  $j = 1, \dots, n$  is

$$y_{ij} = F(x_j, \boldsymbol{\theta}) + \epsilon_{ij}, \quad (1)$$

where  $\boldsymbol{\theta}$  = parameter vector. The errors  $\epsilon_{ij}$  are assumed to be normally, independently distributed with mean zero, constant variance  $\sigma_\epsilon^2$ , and  $cov(\epsilon_j, \epsilon_{j'}) = 0$ ,  $j \neq j'$ .

The functions  $F$  in (1), parameterized for inflection point, are:

$$\text{Logistic, } F(x_j, \boldsymbol{\theta}) = \frac{\alpha}{\{1 + \exp[\gamma(\beta - x_j)]\}}, \quad (2)$$

$$\text{Gompertz, } F(x_j, \boldsymbol{\theta}) = \alpha \exp\{-\exp[\gamma(\beta - x_j)]\}, \quad (3)$$

$$\text{von Bertalanffy, } F(x_j, \boldsymbol{\theta}) = \alpha \left\{1 - \frac{1}{3} \exp[\gamma(\beta - x_j)]\right\}^3, \quad (4)$$

$\alpha > 0, \gamma > 0, \boldsymbol{\theta} = [\alpha \beta \gamma]'$  and  $x > 0$ . Parameter  $\alpha$  is the asymptote,  $\beta$  is the abscissa of the inflection point and  $\gamma$  is related to the maximum growth rate.

In step 2 random variables  $u_1, u_2$  and  $u_3$  were added to the fixed parameters,  $\alpha, \beta, \gamma$ , respectively, to account for their variations among the subjects. It was assumed that the random variables are normally distributed with null mean vector

and covariance matrix  $\phi_{r \times r}$ ,  $r =$  number of random variables,

$$(u_{1i} \ u_{2i} \ u_{3i})' \sim N(\mathbf{0}, \phi), \quad (5)$$

$$\phi_{3 \times 3} = \begin{pmatrix} \text{var}(u_{1i}) & & \\ \text{cov}(u_{1i}u_{2i}) & \text{var}(u_{2i}) & \\ \text{cov}(u_{1i}u_{3i}) & \text{cov}(u_{2i}u_{3i}) & \text{var}(u_{3i}) \end{pmatrix} = \begin{pmatrix} v_{11} & & \\ v_{12} & v_{22} & \\ v_{13} & v_{23} & v_{33} \end{pmatrix}, \quad (6)$$

$i = 1, \dots, N$  subjects.

The functions (2) to (4) are now:

$$\text{Logistic } F(x_j, \theta_i) = \frac{(\alpha + u_{1i})}{\{1 + \exp[(\gamma + u_{3i})((\beta + u_{2i}) - x_j)]\}}, \quad (7)$$

$$\text{Gompertz } F(x_j, \theta_i) = (\alpha + u_{1i}) \exp\{-\exp[(\gamma + u_{3i})((\beta + u_{2i}) - x_j)]\}, \quad (8)$$

$$\text{von Bertalanffy } F(x_j, \theta_i) = (\alpha + u_{1i}) \left\{1 - \frac{1}{3} \exp[(\gamma + u_{3i})((\beta + u_{2i}) - x_j)]\right\}^3, \quad (9)$$

$\theta_i = [\alpha \ \beta \ \gamma \ u_{1i} \ u_{2i} \ u_{3i}]'$ ,  $i = 1, \dots, N$  subjects,  $j = 1, \dots, n$  times.

The mixed models can be fitted with only one random variable,  $u_1$ , or  $u_2$ , or  $u_3$ , with two variables,  $u_1$  and  $u_2$ ,  $u_1$  and  $u_3$ , or  $u_2$  and  $u_3$ , and the complete model with  $u_1$ ,  $u_2$  and  $u_3$ . In step 2 we take into account the variations among individuals of the population, choosing the random variables in the model, in order to have a good prediction of response.

In these steps 1 and 2, the criteria used to choose the model that best described the growth were residual mean squares (rms), the biological meaning of parameter estimates and the convergence in the iteration process of adjustment. In the step 2 the best mixed model, adjusted to each function (logistic, Gompertz and von Bertalanffy), was determined by the likelihood ratio test. The test verifies the null hypotheses, when the models are hierarchical:

$$H_0: \text{models with one random variable, } u_1, \text{ or } u_2, \text{ or } u_3 = \text{fixed model.} \quad (10)$$

$$H_0: \text{models with two random variables} = \text{models with one random variable.} \quad (11)$$

$$H_0: \text{model with three random variables} = \text{models with two random variables.} \quad (12)$$

Alternative hypotheses are one-sided,  $H_a$ : alternative model is better than null model. The probability distribution of the test is approximately a chi-squared distribution, with degrees of freedom equal to the number of parameters of alternative model minus the number of parameters of null model. Only the model chosen in these steps will be analyzed in the step 3.

In the step 3 we added dummy variables to the model chosen in the earlier steps and a multivariate model was fitted to the response data. The fixed parameters  $\alpha$ ,

$\beta$  and  $\gamma$  could be functions of the factors of the experimental design. For instance, the value of the asymptote of the  $i$ th subject,  $\alpha_i$ , will be the average value  $\alpha$ , plus the effects of treatments that characterize the individual. In a factorial experiment, for instance, the comparisons aim to answer the questions: “How did one factor influence the parameters?” and “Considering a determined level of a factor, how is the influence of the another factor in these parameters?” These questions refer to the main effect of factors and to the interaction between factors, respectively, in the analysis of the experiment. In a factorial experiment, where the treatments have the same number of replicates, with two factors,  $a$  levels of factor  $A$  and  $b$  levels of factor  $B$ , the coefficients of orthogonal contrasts for main effect of factor  $A$  and for interaction effect of factor  $B$  in each level of  $A$  can be formulated as follows.  $A_i$  = effect of the  $i$ th level of factor  $A$ ,  $i = 1, \dots, a$ ;  $B_j$  = effect of the  $j$ th level of  $B$ ,  $j = 1, \dots, b$ .

Table 1 - Coefficients of orthogonal contrasts for comparison of  $A$  levels (main effect)

Contrasts	$A_1$			$A_2$			$A_3 \dots A_{a-1}$			$A_a$		
	$B_1$	...	$B_b$	$B_1$	...	$B_b$	$B_1$	...	$B_b$	$B_1$	...	$B_b$
$C_1$	$a-1$	...	$a-1$	$-1$	...	$-1$	$-1$	...	$-1$	$-1$	...	$-1$
$C_2$	$0$	...	$0$	$a-2$	...	$a-2$	$-1$	...	$-1$	$-1$	...	$-1$
...												
$C_{a-1}$	$0$	...	$0$	$0$	...	$0$	$0$	...	$0$	$1$	...	$1$

The sum of the coefficients of each contrast is zero; for instance in  $C_1$ ,  $b(a-1) + b(a-1)(-1) = 0$ . The sum of the product of the coefficients for each pair of contrasts is zero, that is, the contrasts are orthogonal; for instance  $C_1$  is orthogonal to  $C_2$ :  $b(a-1)(0) + b(a-2)(-1) + b(a-2)(-1)(-1) = 0$ . (Table 1).

Table 2 - Coefficients of orthogonal contrasts for comparing  $B$  levels within each  $A$  level (interaction effect)

Contrasts	$A_1$						$A_2 \dots A_a$					
	$B_1$	$B_2$	$B_3$	...	$B_{b-1}$	$B_b$	$B_1$	...	$B_b$	$B_1$	...	$B_b$
$C_1$	$b-1$	$-1$	$-1$	...	$-1$	$-1$	$0$	...	$0$	$0$	...	$0$
$C_2$	$0$	$b-2$	$-1$	...	$-1$	$-1$	$0$	...	$0$	$0$	...	$0$
...												
$C_{b-1}$	$0$	$0$	$0$	...	$1$	$-1$	$0$	...	$0$	$0$	...	$0$

The other orthogonal contrasts are constructed in the same way. (Table 2).

Considering the bivariate model, the observations  $y_{ijl}$  for the  $i$ th subject,  $i = 1, \dots, N$ , at time  $j$ ,  $j = 1, \dots, n$ , for the dependent variables,  $l = 1, 2$  are represented

in the matrix  $\mathbf{y}_{i(n \times l)}$  as follow:

$$\mathbf{y}_{i(n \times 2)} = \begin{pmatrix} y_{i11} & y_{i12} \\ y_{i21} & y_{i22} \\ \dots & \dots \\ y_{in1} & y_{in2} \end{pmatrix} = \begin{pmatrix} f(\theta_i, x_i, cov_i)_{i11} & f(\theta_i, x_i, cov_i)_{i12} \\ f(\theta_i, x_i, cov_i)_{i21} & f(\theta_i, x_i, cov_i)_{i22} \\ \dots & \dots \\ f(\theta_i, x_i, cov_i)_{in1} & f(\theta_i, x_i, cov_i)_{in2} \end{pmatrix} + \begin{pmatrix} e_{i11} & e_{i12} \\ e_{i21} & e_{i22} \\ \dots & \dots \\ e_{in1} & e_{in2} \end{pmatrix}, \quad (13)$$

where  $\mathbf{f}(\theta_i, \mathbf{x}_i, \mathbf{cov}_i)$  and  $\mathbf{e}_i$  are matrices  $n \times l$  of terms expectation value and error, respectively; in (13)  $f$  is a function with parameter vectors  $\theta_i$ , independent variable  $x_i$ , and covariates  $cov_i$ ;  $\mathbf{x}_{i(n \times l)}$  is a matrix of the values of independent variable of the  $i$ th subject. The  $q = 6$  fixed parameters are represented as

$$\boldsymbol{\beta}_{(6 \times 1)} = (\alpha_1, \beta_1, \gamma_1, \alpha_2, \beta_2, \gamma_2)' \quad (14)$$

and the  $r = 6$  random effects for the  $i$ th subject and  $l = 1, 2$  as

$$\boldsymbol{\gamma}_i(6 \times 1) = (u_{1i,l=1}, u_{2i,l=1}, u_{3i,l=1}, u_{1i,l=2}, u_{2i,l=2}, u_{3i,l=2})' \quad (15)$$

The parameter vector  $\theta_i$  is obtained with (14) and (15)

$$\theta_i(12 \times 1) = (\alpha_1, \beta_1, \gamma_1, \alpha_2, \beta_2, \gamma_2, u_{1i,1}, u_{2i,1}, u_{3i,1}, u_{1i,2}, u_{2i,2}, u_{3i,2})' \quad (16)$$

It is assumed that  $\boldsymbol{\gamma}_i \sim N(\mathbf{0}, \boldsymbol{\phi}_{r \times r})$ , with

$$\boldsymbol{\phi}_{6 \times 6} = \begin{pmatrix} v_{11.11} & & & & & & \\ v_{12.11} & v_{22.11} & & & & & \\ v_{13.11} & v_{23.11} & v_{33.11} & & & & \\ v_{11.12} & v_{21.12} & v_{31.12} & v_{11.22} & & & \\ v_{12.12} & v_{22.12} & v_{32.12} & v_{12.22} & v_{22.22} & & \\ v_{13.12} & v_{23.12} & v_{33.12} & v_{13.22} & v_{23.22} & v_{33.22} & \end{pmatrix} \quad (17)$$

where  $v_{kk'l'l'} = cov(u_{k,l}, u_{k',l'})$ ,  $u_{k,l}, u_{k',l'}$  = random variables associated to the fixed parameters  $k, k' = 1, 2, 3$ , and dependent variables  $l, l', = 1, 2$ .

It is assumed also that  $\mathbf{e}_i \sim N(\mathbf{0}, \mathbf{R}_i)$ ,  $\mathbf{R}_i$  with dimension  $2n \times 2n$ . The  $\mathbf{R}$  structure assumes that each line of the matrix  $\mathbf{e}_i$  has variances  $\sigma_{l=1}^2$  and  $\sigma_{l=2}^2$  and covariance  $\sigma_{1,2}$ ; the other terms are uncorrelated.

The methodology is applied to the results of experiments related to the performance of sweet orange trees *Citrus sinensis* (L.) Osbeck, analysed by Ary A. Salibe, in his Thesis Full Professors "Effect of rootstock and locality in the vigor and production of sweet orange trees, *Citrus sinensis* (L.) Osbeck". The experiment consisted of five scions varieties budded on five rootstocks, following a randomized block design, with six replications. Scions varieties were Hamlin (H), Baianinha Navel (B), Westin (W), Rubi (R) and Itaborai (I) oranges. The rootstocks were Rangpur lime *Citrus limonia* Osbeck (LC), Sunki mandarin *Citrus sunki* Hort. ex Tanaka (SU), Caipira sweet orange *Citrus sinensis* (L.) Osbeck (CA), trifoliolate orange *Poncirus trifoliata* Rafinesque (TR) and Florida rough lemon *Citrus jambhiri* Lushington (RF). The experiments were located in Lageado Experiment

Station, Botucatu (latitude 22° 50' 48" S, longitude 48° 26' 06" W and altitude of 786 meters). Yearly, in the period of 1968 to 1974, the orange production was controlled in kilograms of fruits per tree and measurements of trunk circumference in centimeter were registered as an indicative of tree vigor.

PROC MODEL in SAS (STATISTICAL ANALYSIS SYSTEM, version 9.3) for nonlinear functions was used to fit the models to each subject in step 1. The errors assumptions were verified with Durbin-Watson, Breusch-Pagan and Shapiro-Wilk tests, for independence, homoscedasticity and normality, respectively. PROC NLMIXED in SAS was used in step 2, and in step 3 was adapted for multivariate nonlinear mixed models (Strathe et al., 2010).

### 3 Results and discussion

In the citrus experiment the subjects were the plants with 5 scions, 5 rootstocks and 6 replicates; there were  $N = 150$  plants. The age of the trees was measured in years,  $x_j = 6, 7, 8, 9, 10, 11$  years,  $n = 6$ . All the plants with Itaboraí scion were excluded from the analysis of the orange production (OP) data since the observations along time do not follow a s-shaped curve; in this case,  $N = 120$  plants. In the steps 1 and 2 there were  $N = 150$  plants for the trunk circumference (TC) data. In step 3,  $N = 120$  plants were considered for both variables, OP and TC.

*Results for the OP data:* In step 1 the logistic model fitted all the 20 combinations cup-rootstock, and Gompertz, 18. The iterative process of adjustment did not converge in three combinations for von Bertalanffy function, and in other four combinations the parameter estimates were without biological meaning, with the asymptotes overestimated. The residual mean square values were 465.49, 512.31 and 625.80 for logistic, Gompertz and von Bertalanffy functions, respectively. In this step the logistic model had a better fit than Gompertz and von Bertalanffy models, with the smaller average value of residual mean square, and 100% of convergence; asymptote estimates and coefficient of variation in brackets were 297.8 (40), 447.5 (54) and 556.5 (42) in average, for logistic, Gompertz and von Bertalanffy functions, respectively. The average observed production at 11 years was 197.4 kg. On average for the three functions, the assumptions for the errors  $\epsilon_{ij}$  in (1) were rejected in nearly 50% of the cases (significance level = 0.05).

In step 2 the likelihood ratio test showed the best performance of model  $u_{123}$  in comparison with the fixed and other mixed models. For all the logistic models the p-values for the test of the hypotheses (10), (11) and (12) were lower than 0.0001. For the Gompertz models, the hypothesis  $H_0 : u_3 = \text{fixed model}$  was not tested, once the  $u_3$  model is worse than the fixed model; the test for the hypothesis  $H_0 : u_{23} = u_2$  had p-value 0.00288 and for  $H_0 : u_{123} = u_{12}$ , 0.25087. For the other hypotheses, p-values were lower than 0.0001. For the von Bertalanffy model, four hypotheses were discarded because the alternative model was not as well fitted as the null model:  $H_0 : u_3 = \text{fixed}$ ,  $H_0 : u_{12} = u_2$ ,  $H_0 : u_{13} = u_1$  and  $H_0 : u_{13} = u_3$ . The p-value for testing the hypothesis  $H_0 : u_{23} = u_2$  was 0.00035; the other tests showed

p-values lower than 0.0001. The values of y-variance, 189.88, 384.33 and 440.14, and corrected Akaike information criterion, 6619.5, 6720.0 and 6737.3, obtained in the  $u_{123}$  mixed model for logistic, Gompertz and von Bertalanffy models, respectively, were lower in the logistic in comparison with the other two functions.

*Results for the TC data:* According to the criteria used here, the models adjusted to the TC data showed very similar results between themselves. In step 1 the logistic and Gompertz models fitted all the 25 cup-rootstock combinations and the von Bertalanffy had three cases with asymptote overestimated. The three functions had similar adjustments, according to the residual mean square criterion; however the asymptote estimate in logistic (61.5 cm) is nearer from the average observed trunk circumference value at 11 years, 47.7 cm, in comparison with the Gompertz (81.6) and von Bertalanffy (70.5) estimates. Except for von Bertalanffy model  $u_2$ , that adjusted worse than the fixed model, all hypotheses verified with the likelihood ratio test resulted in p-values lower than 0.0001 in step 2. Therefore, the  $u_{123}$  model can be chosen as the best mixed model. The Akaike information criterion and the y-variance(vy) values for the  $u_{123}$  model are similar in the three functions. In the OP analysis the y-variance of logistic  $u_{123}$  model was 56% smaller than the Gompertz similar model and 57% smaller than the von Bertalanffy model. Considering that the logistic mixed  $u_{123}$  model fits better for the OP data, we chose this model for the subsequent analyses in the step 3.

*Step 3.* In the bivariate logistic model (13), only the random variables in the asymptotes related to OP and TC data,  $u_{1.1}$  and  $u_{1.2}$  respectively, were used; other models with parameters of vector (16) did not converge. The vector  $\theta$  in (16) was

$$\theta_{i(8 \times 1)} = (\alpha_1, \beta_1, \gamma_1, \alpha_2, \beta_2, \gamma_2, u_{1i.1}, u_{1i.2})' , \quad (18)$$

and the covariance matrix  $\phi$  in (17) was reduced to

$$\phi_{3 \times 3} = \begin{pmatrix} v_{11.11} & & \\ & v_{11.12} & \\ & & v_{11.22} \end{pmatrix}. \quad (19)$$

In this step, TC data were used in millimeters, in order to avoid great differences between the initial values of the OP and TC  $\alpha$  parameters, what can lead to problems of convergence in the iterative process in SAS. The contrasts were:

Rootstock effect in the asymptote considering each scion,

$$LC + SU \text{ vs. } TR = 1(LC) + 1(SU) + 0(CA) - 2(TR) + 0(RF) \quad (20)$$

Rootstock effect in the abscissa of the inflection point considering each scion,

$$LC \text{ vs } CA = 1(LC) + 0(SU) - 1(CA) + 0(TR) + 0(RF) \quad (21)$$

Scion main effect in the asymptote and abscissa of inflection point,

$$H \text{ vs other scions} = 3(H) - 1(B) - 1(W) - 1R \quad (22)$$

With the significance level  $\alpha = 0.05$  it was observed: the rootstock influence on the asymptote of the logistic model (13), measured by the

$LC + SU - 2TR$  contrast, was significant for the OP observations in the cups H, B, and R and for the TC observations in all the cups. As the contrast estimates are positive, we conclude that LC and SU rootstocks induced expected superior limits of fruit yield and trunk circumference higher than the TR rootstock; the conclusion can be observed in the Figure 1 for the scion Hamlin. The scion effect on the asymptote, measured by the contrast  $3H - B - W - R$ , is significant and positive in the average of all rootstocks; the Hamlin (H) growth curves of OP and TC data had higher asymptote than the other cups B, W and R. This conclusion can be observed in the Figure 2.

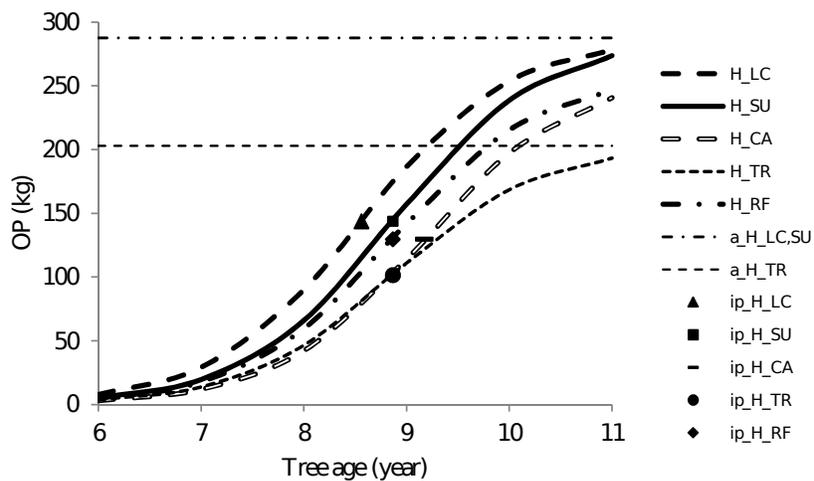


Figure 1 - Rootstock effects on the asymptote,  $LC + SU - 2TR$ , and on the abscissa of the inflection point,  $LC - CA$ , of the growth curves of trees with Hamlin (H) cup, fitted to orange production (OP) data. LC: Rangpur lime; SU: Sunji mandarin; CA: Caipira sweet orange; TR: Trifoliate orange; RF: Florida rough lemon; a: asymptote; ip: inflection point. Model (13).

Rootstock effect on the abscissa of the inflection point in model (13), measured by the contrast  $LC - CA$ , is negative and significant for the OP and TC observations in all the cups H, B, W and R; trees with these cups budded on LC rootstock reached the inflection point before than trees budded on CA rootstock. This is shown in the Figure 1. The scion effect on the abscissa of the inflection point, measured by the contrast  $3H - B - W - R$  for all the rootstocks, is significant and negative for OP and positive for TC data, ie. Hamlin trees are more precocious in obtaining great productions when compared with the B, W and R trees, but reach the maximum point of growth velocity in trunk circumference after the other cups. Figure 2.

The mixed model (13), with corrected Akaike information criterion (AICc)

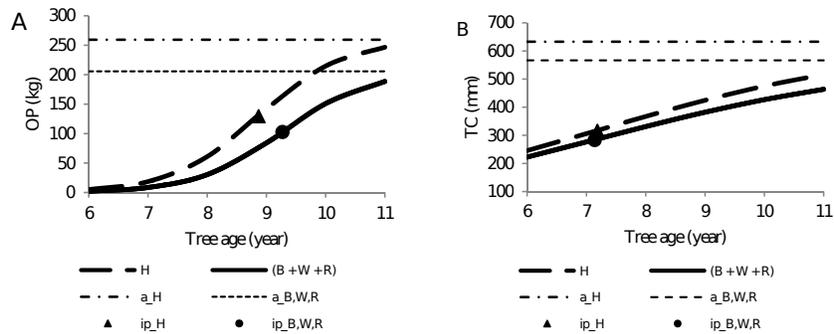


Figure 2 - Cup effect,  $3H - (B+W+R)$ , on the asymptote and abscissa of inflection point of the growth curves of trees for the five rootstocks. A: orange production (OP) data; B: trunk circumference (TC) data; H: Hamlin; B: Baianinha; W: Westin; R: Rubi; a: asymptote; ip: inflection point. Model (13).

value of 500888, represented better the population in comparison with the fixed model, with AICc of 717379; the likelihood ratio test had p-value lower than 0.0001. Figure 3 presents OP residuals from logistic fixed model (2) and from logistic mixed bivariate model (13); in the latter, the residuals are smaller and more homogeneous than in the former. This shows that the residuals in the model (13) are more in agreement with the assumptions in (1) than the residuals in the fixed model (2).

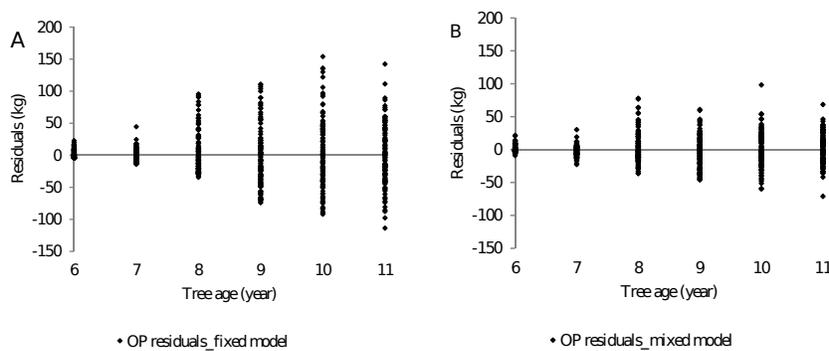


Figure 3 - Orange production (OP) residuals from logistic. A: fixed model (2) and B: mixed bivariate model (13).

## Conclusions

The nonlinear mixed multivariate logistic model with orthogonal contrasts introduced as covariates can be efficiently used in determining differences between parameters according to main effects and interactions in factorial experiments. The multivariate mixed model can contribute more than the univariate model.

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- **RESUMO:** *O objetivo desse estudo foi estabelecer contrastes em modelos mistos não lineares multivariados para verificar o efeito de tratamentos em experimentos com dados longitudinais e respostas múltiplas. As funções não lineares avaliadas foram logística, Gompertz e von Bertalanffy, todas com três parâmetros. As variáveis aleatórias foram adicionadas aos parâmetros assíntota  $\alpha$ , abscissa do ponto de inflexão  $\beta$  e o parâmetro  $\gamma$ . O melhor modelo ajustado foi expandido com covariáveis que estabelecem contrastes ortogonais, de modo a estudar os efeitos principais e interações em experimentos fatoriais. A metodologia foi aplicada a dados de um experimento com citros, onde o modelo logístico de efeitos mistos bivariado foi o melhor ajuste. O modelo escolhido permitiu comparações entre os tratamentos em um contexto global de mais de uma variável dependente e ao longo do período de mensuração.*
- **PALAVRAS-CHAVE:** *Regressão não linear; modelos assintóticos; variáveis dummy; dados de citros.*

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