LONGITUDINAL DATA ANALYSIS OF *STEVIA REBAUDIANA* EVAPOTRANSPIRATION ACCORDING TO WATER LEVELS

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- ABSTRACT: The purpose of this work was to evaluate the accumulated evapotranspiration of stevia, planted in 40 pots, over a period of 27 days according to four irrigation levels through a nonlinear mixed effects model and, with this model, to verify if we can maximize the stevia's evapotranspiration with a lower than usual water level. When the humidity of the substrate reached 50% of its maximum capacity retention, we added water to raise the humidity according to the four treatments: $W_1 = 62.5\%$; $W_2 = 75.0\%$; $W_3 = 87.5\%$; $W_4 = 100.0\%$. Although the crop production depends on the availability of water in the soil, there is a limit for the total yield. The water levels, W_3 and W_4, resulted in similar total accumulated evapotranspiration, while all the last three water levels, W_2, W_3 and W_4 had close shoot dry matter masses at the end of the experiment. We showed that is possible to find an optimal level of crop production with a rational use of the available water resources.

- KEYWORDS: Irrigation; growth curve; microlysimeter; nonlinear mixed effects model; potable water savings.

1 Introduction

According to the ONU report (CONNOR, 2015), growth of the global population and water consumption will cause a decrease in water availability *per capita* and in water quality, requiring a more sustainable use of the available water

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resources. The human population is expected to grow three times more than the potable water supply over this century (VÖRÖSMARTY *et al.*, 2000; POSTEL *et al.*, 1996). This increase and changes in income and diets will also make the demand for food to almost double (TILMAN *et al.*, 2011; GREGORY and GEORGE, 2011).

In the world, irrigated agriculture represents 70% of fresh water withdrawals (ASSOULINE *et al.*, 2015). It is the most efficient way to increase crop yields per unit land. About 30% of crop production is obtained with irrigated agriculture (HARGREAVES and SAMANI, 1982; ALLEN *et al.*, 1998; FERERES and SORIANO, 2006; HOWELL, 2001; ENGLISH *et al.*, 2002).

Prolonged droughts and heavy rainfalls such as the flood in Amazonia and the drought in Southeast and Northeast Brazil in the last years severely affected Brazilian agricultural and irrigation water supply (GETIRANA, 2016), causing massive financial losses. As these extreme events increase and agriculture becomes more intensive, more efficient use of the available water, in terms of both quantity and quality, is necessary in the sustainability of irrigated lands.

It is known that evapotranspiration depends mainly on soil moisture in most situations. If the irrigation level is too high (low), i.e, greater (smaller) than the potential evapotranspiration, the growth and development of plants may not reach their full potential. Thus, one way for enhancing irrigation is knowing what amount of water is optimum for maximizing crop yields.

During growth and development of crops, large amounts of water are required for the plants to carry out their metabolic processes. About 95% of absorbed water by the plant is transpired in order to maintain the transport of nutrients, turgidity and leaf cooling. Physical factors such as solar radiation, temperature, relative humidity, wind speed and especially the water availability in the soil affect evapotranspiration. Changing these factors, for example, diminishing the amount of water damages the growth and development of plants (ZHAO *et al.*, 2013; HANKS *et al.*, 1969; KIRDA, 2002; ZHANG *et al.*, 2011; JAIN *et al.*, 2008; FAN and THOMAS, 2013; ALLEN *et al.*, 2011).

The aim of this work was to estimate the evapotranspiration of *Stevia rebaudiana* (Bert.) Bertoni according to four water deficit levels with a nonlinear mixed effects model. Stevia has been cultivated in several countries because of the sweetness of the leaf extract, which has a sweetener power 300 times greater than sugar-cane, but has no caloric value.

2 Materials and methods

The experiment was conducted inside a greenhouse at Maringá State University, Brazil. In each of 40 impermeable black polyethylene pots, three seedlings of *Stevia Rebaudiana* were planted. The pots were randomly assigned to four different water levels treatments. 5000 g of a material derived from Red Latosol, with 30% clay, was collected from a layer of 20 cm soil depth, passed through a sieve of 4 mm and air dried. Its density in the pots were standardized at 1.33 g cm^{-3}. The maximum capacity of water retention of this material in the pot was estimated to be 27.4% of the dry weight substrate.

The treatments began 20 days after the seedlings were transplanted into the pots, by this time, one of the seedlings was removed, remaining two in each pot. When the humidity of the substrate reached 50% of its maximum capacity retention, we added water to raise the humidity according to the four treatments: $W_1 = 62.5\%$; $W_2 = 75.0\%$; $W_3 = 87.5\%$; $W_4 = 100.0\%$. In other words, each treatment was maintained with humidity deficit between 50 and 37.5%, 50 and 25% 50 and 12.5%, and 0 and 50%, respectively. The evapotranspired water mass of each pot was recorded daily for 27 consecutive days with a weighing scale having a maximum weighing capacity of 8 kg and a precision of 0.1 g. Due to the increased fresh weight of the plants throughout the experiment, additional pots were necessary to correct the mass of the pots. At the end of the experiment, the shoot dry matter per pot was measured.

3 Nonlinear mixed effects model

We proposed the following nonlinear mixed effects model for the growth curve of the accumulated evapotranspiration, E_{ij}, per pot i at t_j days after planting:

$$E_{ij} = \phi_{2i} + \frac{\phi_{1i} - \phi_{2i}}{1 + \exp\left[(\phi_{3i} - t_j)/\phi_{4i}\right]} + \epsilon_{ij}, \quad i = 1, \ldots, 40 \text{ and } j = 1, \ldots, 27, \tag{1}$$

where

$$\boldsymbol{\phi}_i = \begin{bmatrix} \phi_{1i} \\ \phi_{2i} \\ \phi_{3i} \\ \phi_{4i} \end{bmatrix} = \begin{bmatrix} \beta_1 + \gamma_1 x_{1i} + \delta_1 x_{2i} + \zeta_1 x_{3i} \\ \beta_2 + \gamma_2 x_{1i} + \delta_2 x_{2i} + \zeta_2 x_{3i} \\ \beta_3 + \gamma_3 x_{1i} + \delta_3 x_{2i} + \zeta_3 x_{3i} \\ \beta_4 + \gamma_4 x_{1i} + \delta_4 x_{2i} + \zeta_4 x_{3i} \end{bmatrix} + \begin{bmatrix} b_{1i} \\ b_{2i} \\ b_{3i} \\ b_{4i} \end{bmatrix} \tag{2}$$

$$= \boldsymbol{\beta} + \boldsymbol{\gamma} x_{1i} + \boldsymbol{\delta} x_{2i} + \boldsymbol{\zeta} x_{3i} + \boldsymbol{b}_i. \tag{3}$$

The parameters $\boldsymbol{\beta}$, $\boldsymbol{\gamma}$, $\boldsymbol{\delta}$ and $\boldsymbol{\zeta}$ represents the fixed effects of the model, with $\boldsymbol{\beta}$ being the reference level, and the $\boldsymbol{b}_i$ the random effects, which are considered to be independent between the pots. The covariables x_{ki}, $k = 1, 2, 3$ indicate the water level that pot i was subjected to, for example, if pot i received the third treatment, then $x_{1i} = 0$, $x_{2i} = 1$ and $x_{3i} = 0$, i.e., $\boldsymbol{\phi_i} = \boldsymbol{\beta} + \boldsymbol{\delta} + \boldsymbol{b}_i$. The within-group errors $\epsilon_{ij} \sim N(0, \sigma^2)$ are assumed to be independent for different i, j and to be independent of the random effects, $\boldsymbol{b}_i \sim N(\mathbf{0}, \boldsymbol{\Psi})$.

This is a four-parameter logistic model where ϕ_{1i} is the horizontal asymptote when $t \to -\infty$, ϕ_{2i} is the horizontal asymptote when $t \to +\infty$, ϕ_{3i} is the t value at the inflection point (which is also the medium point between the asymptotes), and ϕ_{4i}, the scale parameter, corresponds to the time beginning from ϕ_{3i} in which the response is approximately 3/4 of the distance between the asymptotes (PINHEIRO and BATES, 2000).

The analysis was carried out with `R` statistical software (R CORE TEAM, 2017) using package `nlme` (PINHEIRO *et al.*, 2018).

4 Results and discussion

Figure 1 shows on the first graph the observed daily evapotranspiration of the plants in the 40 pots as a function of the four water levels, the plot just bellow shows the daily temperature and precipitation over the period of the experiment. Following the rainy days, it seems that the evapotranspiration values declined, probably due to the increase in relative humidity. However, the temperature does not have a very clear relationship with evapotranspiration.

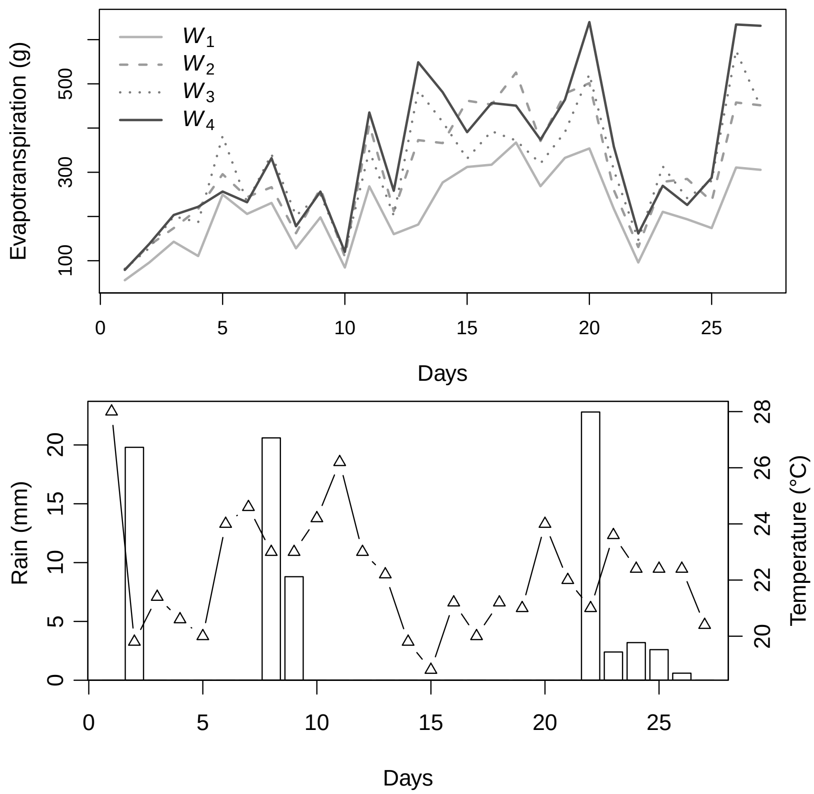

Figure 1 - Upper panel: daily evapotranspiration of *Stevia rebaudiana* according to the four water levels: $W_1 = 62.5\%$; $W_2 = 75.0\%$; $W_3 = 87.5\%$; $W_4 = 100.0\%$. Lower panel: recorded daily temperature (right axis) and precipitation (left axis) over the duration of the experiment.

Our aim is to ascertain the relationship between the parameters of the logistic model, which summarizes the growth curves of the accumulated evapotranspiration, and the factor with four different water levels. First, we fitted model (1) without the covariables and with a general positive-definite $\mathbf{\Psi}$. Indeed, testing nested models with $\gamma_h = \delta_h = \zeta_h = 0$, $h = 1, 2, 3, 4$ results in the rejection, at the 5% signifcance level, of all four of them. Finally, as the inclusion of the covariates may account for intergroup variation, we tested for the elimination of random effects and end up with the model without random effects for the scale parameter ϕ_4. This is the model-building strategy adopted by PINHEIRO and BATES (2000). Also, regarding these authors, the incorporation of the within-group autocorrelation structure may reduce the need for random effects in the model, since one may replace the other with the intent of accommodate the non explained variability of the treatments. Therefore some autocorrelation structures were considered, in special the AR(1), because since the autocorrelation happen over time, it is expected that the evapotranspiration values in adjacent times to be more autocorrelated than observations made in distant times. Besides that, the best configuration we obtained between random effects and within-group autocorrelation structures, was described above, i.e., we adopted a independent autocorrelation structure for the errors and we kept the random effects of the parameters ϕ_1, ϕ_2 e ϕ_3.

The likelihood ratio test was used to compare the models. Besides that, the Akaike and Bayesian information criterion were also used to decide among models. At the general correlation matrix with the additional structure, $\mathbf{\Psi}$, the estimated variances for the random effects b_1, b_2 and b_3 were 0.0042, 0.7320 and 0.1643, and the correlations between b_1 and b_2, b_1 and b_3, and b_2 and b_3 were -0.859, 0.053 and 0.465 respectively. Even though the correlation between b_1 and b_2 are high, the elimination of any of them worsens the model adjustment and, therefore, in the final model, we kept the three random effects.

The estimated values for the fixed effects together with their respective standard errors are in Table 1. Our main interest relies on parameters ϕ_2 and ϕ_3, the other parameters, ϕ_1 and ϕ_4, we treated as nuisance parameters. Parameter ϕ_2 is the accumulated evapotranspiration when $t \to +\infty$. Note that the estimated effect $\hat{\delta}_2$, the effect of the water level W_3 on the parameter ϕ_2, is larger than $\hat{\zeta}_2$, although the associated standard errors are high. Furthermore, ϕ_3, which represents the day at the inflection point, does not vary much across the water levels. Beside these parameters, we must discuss about ϕ_1, the lower asymptote. Its presence in the model allows a better adjustment for the smaller values of the accumulated evapotranspiration regarding the firsts days of experiment. If the parameter were not put in the model, theoretically we would only have zeros for the accumulated evapotranspiration when $t \to -\infty$, which may not correspond to the reality. We observed, for example, in the Table 1 that the reference value for ϕ_1, the $\widehat{\beta}$ parameter, is negative. This allows for the adjusted curve to estimate smaller or near zero values, when crossing time equals zero.

The resulting estimated curves together with the observed values of the accumulated evapotranspiration can be seen in Figure 2. Each plot in the figure corresponds to a water level. The thick black line is the marginal model, i.e., the population mean curve. The other continuous lines are the conditional models that account for the random effects, i.e., the individual models for each observed curve. For all treatments, the model predictions are very close to the observed

Table 1 - Estimated values for the fixed effects, according to Equation (3), together with their respective standard errors in parentheses

	$\widehat{\boldsymbol{\beta}}$ W_1	$\widehat{\boldsymbol{\gamma}}$ W_2	$\widehat{\boldsymbol{\delta}}$ W_3	$\widehat{\boldsymbol{\zeta}}$ W_4
ϕ_1	-651 (74.07)	6 (103.69)	-1233 (193.96)	-290 (112.36)
ϕ_2	7270 (306.16)	2442 (428.25)	4580 (549.51)	3847 (441.21)
ϕ_3	16.8 (0.25)	-0.11 (0.31)	1.19 (0.44)	0.45 (0.32)
ϕ_4	7.1 (0.23)	-0.7 (0.27)	2.38 (0.47)	-0.15 (0.29)

values, indicating a great fit of the growth curves by the nonlinear mixed effects model. Observe that treatment W_1, when compared to the other water levels, the mean curve is clearly inferior and the individual curves present great variability, so the final results depend more on the specific characteristics related to the genetics of each plant. Once enough irrigation is applied, treatments W_3 and W_4, the behavior of the accumulated evapotranspiration becomes more homogeneous and more independent of the individual characteristics.

Also, in Figure 2, it is possible to observe that, throughout the estimated curves, there are small systematic oscillations of the observed values. It is highly due to the variation of the relative humidity over time as observed in Figure 1. This variations at the daily evapotranspiration certainly had an impact in the accumulated evapotranspiration causing the listed effect. The proposed model enable the adjustment of a mean curve for the data, not considering the observations systematic oscillations.

Diagnostic graphs for the four-parameter logistic model used are displayed in Figure 3. The first two plots from left to right show the standardized residuals *versus* the estimated values and the observed values *versus* the estimated values (the straight line represents a perfect fit). They do not indicate large deviations from the proposed nonlinear model. The last plot is a quantile-quantile (Q-Q) graph for the assumption of normal distribution of the residuals. The linearity of the points suggests no serious violation of this assumption.

More evapotranspiration translates to more dry matter. We investigated this relation in Figure 4, where the shoot dry matter per pot is plotted against the total accumulated evapotranspiration observed in the period of 27 days. The symbols for the points in the graph represent the water levels. There is a visible linear relationship between these two variables. A simple linear regression fitted to this data provides an slope of 0.003279, meaning that in order to produce one kilo of shoot dry matter, about 300 kilos of total evapotranspirated water are needed. The observed means for the production of shoot dry matter were 15.19 (1.04) g, 23.04 (0.79) g, 24.47 (0.93) g and 26.06 (0.90) g for treatments W_1, W_2, W_3 and W_4. Note that W_2, W_3 and W_4 have very similar means and standard errors, and each of these treatments has an observation that is more distant from the mean.

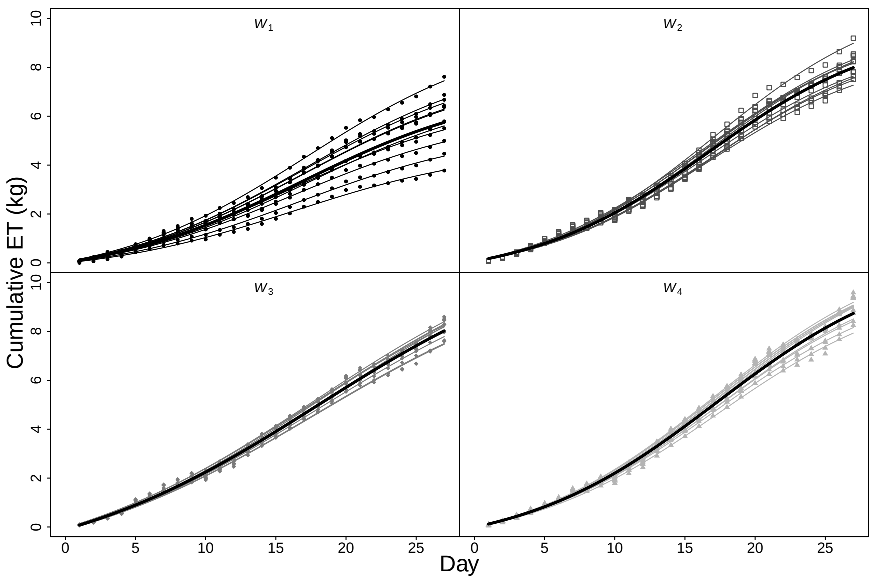

Figure 2 - Observed and predicted growth curves for the accumulated evapotranspiration of *Stevia rebaudiana* according to the four water levels: $W_1 = 62.5\%$; $W_2 = 75.0\%$; $W_3 = 87.5\%$; $W_4 = 100.0\%$. The thick black line is the marginal model and the other continuous lines are the conditional models that account for the individual effects.

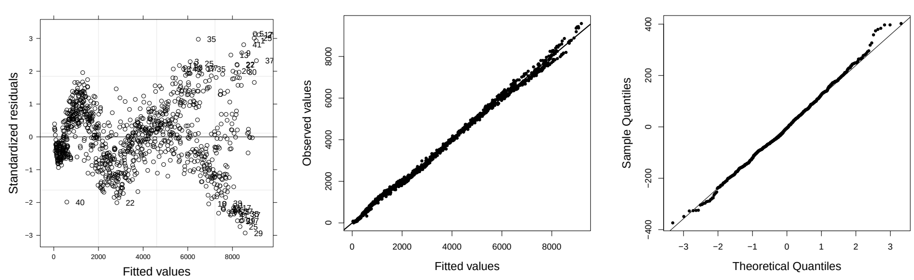

Figure 3 - Diagnostic graphs. From left to right: standardized residuals *versus* estimated values; observed values *versus* estimated values; normal Q-Q plot for the residuals.

5 Final considerations

The nonlinear mixed effects model we adopted fitted very well to the accumulated mass of evapotranspirated water over the period of 27 days for the

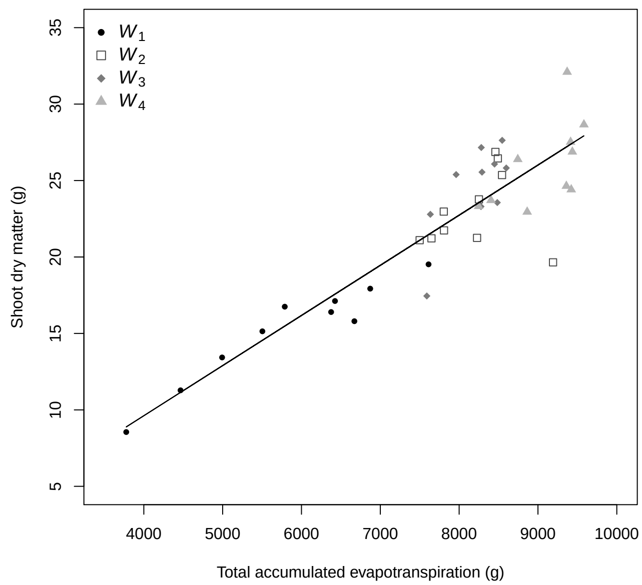

Figure 4 - Shoot dry matter of the *Stevia rebaudiana* per pot *versus* total accumulated evapotranspiration according to the four water levels: W_1 = 62.5%; W_2 = 75.0%; W_3 = 87.5%; W_4 = 100.0%, and a fitted line of a simple linear regression.

Stevia rebaudiana plants in the 40 pots, and provided a good representation of the data. The individual accumulated evapotranspiration curves (conditional curves) had more variability around the population curve (marginal model) for W_1 and W_2, indicating that a proper irrigation level produces more homogeneous growth curves (Figure 2). One possible explanation for this phenomenon is that for low water levels, factors like the genetic variability of the plants become more evident. Moreover, the estimated superior asymptotic level of the growth curves were higher for W_3 and W_4.

The treatments corresponding to last three water levels, $W_2 = 75.0\%$; $W_3 = 87.5\%$; $W_4 = 100.0\%$, produced similar shoot dry matter masses. Thus, it is possible to obtain high productions of shoot dry matter with a more rational use of the water resources.

In agricultural experiments where pots are used as experimental units, the manner in which irrigation is done is often neglected, even though water is the main input in crop production. Most of these experiments do not describe how the reposition of water are made in the pots, some studies do this for simple omission and others for not having used any appropriate method. For example, in this experiment with *Stevia rebaudiana*, the observed shoot dry matter for the pots that reached the lowest and highest evapotranspiration, 3777 g and 9583 g of water, were 8.88 g and 27.93 g. The difference between these amounts of evapotranspirated water was 5086 g, giving a mean of 215 g/day. So a small daily increase in the amount of irrigated water is enough to cause great differences in the production of dry matter. Therefore, a poor planned irrigation method in agricultural experiments could provide misleading results.

This study shows that the increase in crop production is highly dependent on the amount of water available in the soil, and that high levels of irrigation are not necessary to maximize the production. It is important to balance the amount of water used, searching for an optimal level, so that the production is not compromised and valuable water resources are not wasted.

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- *RESUMO: O objetivo deste trabalho foi avaliar a evapotranspiração acumulada da stevia, plantadas em 40 vasos, durante um período de 27 dias em função de quatro níveis de água por meio de um modelo com efeitos mistos não-linear e, com este modelo, verificar se podemos maximizar a evapotranspiração da stevia com um nível de água menor que o usual. Quando a umidade do substrato atingia 50% de sua capacidade máxima de retenção, adicionava-se água para elevar a umidade de acordo com os quatro tratamentos:* $W_1 = 62,5\%$*;* $W_2 = 75,0\%$*;* $W_3 = 87,5\%$*;* $W_4 = 100,0\%$*. Embora a produção agrícola dependa da disponibilidade de água no solo, há um limite para o rendimento máximo. Os níveis de água* W_3 *e* W_4*, resultaram numa evapotranspiração acumulada total similar, enquanto que todos os três últimos níveis de água,* W_2*,* W_3 *e* W_4 *tiveram massas de matéria seca de parte aérea próximas ao final da experiência. Mostrou-se que é possível encontrar um nível ótimo de produção agrícola com uso racional dos recursos hídricos disponíveis.*

- *PALAVRAS-CHAVE: Curva de crescimento; economia de água potável; irrigação; microlisímetro; modelo com efeitos mistos não-linear.*

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