

## LIMIT BETWEEN STAGES I AND II OF A SEED IMBIBITION CURVE

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**ABSTRACT:** A knowledge of the imbibition stages shown by different species, is essential in research projects aiming at improvement of seed quality by treatments such as osmotic conditioning, initial wetting, and the use of bioregulators. The objective of this study was to establish a methodology to determine the limit between the first and second stages of the process, considering the model  $W = f(t) - (a - w_0)\exp(-kt)$ , using statistical tests. This limit was determined using an asymptotic distribution of an estimator function. The data point beyond which the difference ( $W^*$ ) is no longer significant was determined using Student's T statistics. The model is  $\hat{W} = (0.434 + 0.00162 t) - (0.434 - w_0)\exp(-0.121 t)$ , with  $r^2 = 0.98$  and  $W^* = (0.434 - w_0)\exp(-0.121 t)$  fits the utilized data. The calculated t value (27.2 hours) is smaller than the value that was determined considering a 1% value difference between the asymptote and the estimated model as a criterion for the stage change. This two-hour difference corresponds to 0.28% in absorbed water.

**Key words:** water absorption, non-linear regression, germination

## LIMITE ENTRE AS FASES I E II DE UM MODELO DE EMBEBIÇÃO DE SEMENTES

**RESUMO:** O conhecimento das fases de absorção de água das diferentes espécies é importante em pesquisas objetivando melhorar a qualidade das sementes, utilizando tratamentos como condicionamento osmótico, pré-hidratação e uso de bioreguladores. O objetivo do presente trabalho foi estabelecer uma metodologia para determinar o limite entre a primeira e a segunda fase do processo, considerando o modelo  $W = f(t) - (a - w_0)\exp(-kt)$ , utilizando testes estatísticos. O limite entre as duas primeiras fases do processo de absorção de água pelas sementes, foi determinado utilizando-se a distribuição assintótica de uma função de estimadores. O ponto a partir do qual esta diferença ( $W^*$ ) deixa de ser significativa foi determinado utilizando-se o teste estatístico T de Student. Para os dados utilizados como exemplo, tem-se o modelo  $\hat{W} = (0,434 + 0,00162 t) - (0,434 - w_0)\exp(-0,121 t)$ , com  $r^2 = 0,98$  e  $W^* = (0,434 - w_0)\exp(-0,121 t)$ . O valor de t encontrado (27,2 horas) é menor do que o valor determinado, considerando-se como critério para mudança de fase a diferença de 1% entre a assíntota e o valor estimado pelo modelo ajustado. Essa diferença de duas horas corresponde a 0,28% de água absorvida.

**Palavras-chave:** absorção de água, regressão não-linear, germinação

### INTRODUCTION

Seed germination can be defined as a series of metabolic and morphogenetic processes, which result in the transformation of the embryo into a seedling capable of becoming an adult plant. Sequential and synchronized processes occur during germination, and are organized in such a way that the anabolic and catabolic processes take place simultaneously (Coll et al., 1995).

Seeds with a permeable integument exhibit a three-stage water absorption process (Bradford, 1995), consisting of imbibition (stage I), activation or germination *sensu stricto* (stage II), and growth (stage III). Stage II is sensitive to factors that influence germination time and amplitude, when the water content in the seed gradually increases or remains unchanged, and then

suddenly increases as the emerging radicle starts to grow; this stage ends as growth begins (Bewley & Black, 1978).

The duration of each stage (Bewley & Black, 1994) depends on properties inherent to the seeds, such as the level of hydratable substrates, integument permeability, seed size, and oxygen absorption. It also depends on the hydration conditions, such as temperature, moisture level, and substrate composition.

The rapid initial absorption is a consequence of the water potential difference between the seed and the substrate, which is caused by the high matrix potential of the seed. This water movement always occurs spontaneously, according to a decreasing water potential gradient (Villega et al., 1991), and is qualitatively the same in either viable or non-viable seeds (Bewley & Black, 1994).

Seed imbibition studies have shown the influence exercised by species, seed age, and the environment conditions on the establishment of a model that would properly represent this process until the initial germination stage. Most reports present models that represent the first two stages, as in Blacklow (1972), Hadas & Russo (1974), Bruckler (1983), Leopold (1983), Bouaziz & Bruckler (1989), Studdert et al. (1994), and Lopes et al. (1996; 2000). Delachiave & Pinho (2001), Almeida & Delachiave (2001), and Braga et al. (2001) presented models that represented the three stages of the process for *Senna occidentalis*, *Leonorus sibiricus*, and *Pachyrhizus ahipa* seeds, respectively.

Blacklow (1972), working with maize (*Zea mays* L.), and Studdert et al. (1994), working with wheat (*Triticum aestivum* L.), described the first two stages of the water absorption process by the equation  $W = f(t) - (a - w_0)\exp(-kt)$ , where  $W$  is the amount of seed water based on its dry weight;  $f(t) = a + bt$  is the asymptote of the exponential curve in which  $a$  is the asymptote value at time  $t = 0$  and  $b$  is the linear slope; and  $w_0$  is the initial amount of seed water.

The  $k$  parameter is related to seed permeability to water,  $a$  relates to the seed initial water capacity, and  $b$  relates to the rate of change in the seed water capacity during the imbibition process. Blacklow (1972) proposed to consider as the end of the first stage, the time necessary for the difference between the asymptote and the value estimated by the model to be equal to 1%. This proposition was also utilized by Studdert et al. (1994).

In research studies aimed at improving seed quality with the use of treatments such as osmotic conditioning, initial wetting and the use of bioregulators, it is important to obtain a knowledge of the water absorption stages for different species. Therefore, the objective of this work was to establish a methodology to determine the limit between the first and second stages of the process, considering the model presented by Blacklow (1972), with the use of statistical tests.

## MATERIAL AND METHODS

To illustrate the methodology and the deduced formulas, one of the models presented by Studdert et al. (1994) was utilized. The seed imbibition data were generated according to the function  $W = (0.44 + 0.0017t) - (0.44 - 0.09)\exp(-0.109t)$ , considering the time interval from zero to 100 hours, at every five hours.

The limit between the first two stages of the process of water absorption by the seeds, based on the model  $W = f(t) - (a - w_0)\exp(-kt)$ , was determined using the asymptotic distribution of an estimator function, as described in Hoffmann & Vieira (1983).

Considering  $a$ ,  $b$  and  $c$  as unbiased estimators of  $\alpha$ ,  $\beta$  and  $\gamma$ , respectively, with  $V(a)$ ,  $V(b)$  and  $V(c)$  their variances and  $d = \phi(a, b, c)$  a function of first- and second-order continuous derivatives in a vicinity of  $a = \alpha$ ,  $b = \beta$  and  $c = \gamma$ , it can be demonstrated that  $d = \phi(a, b, c)$  is a consistent estimator of  $\Delta = \phi(\alpha, \beta, \gamma)$ , with an asymptotically normal distribution of variance

$$\hat{V}(d) = \phi_1^2 \hat{V}(a) + \phi_2^2 \hat{V}(b) + \phi_3^2 \hat{V}(c) + 2\phi_1\phi_2 C\hat{\sigma}_v(a, b) + 2\phi_1\phi_3 C\hat{\sigma}_v(a, c) + 2\phi_2\phi_3 C\hat{\sigma}_v(b, c) \quad (1)$$

where

$$\phi_1 = \frac{\partial\phi(a, b, c)}{\partial a} \quad \phi_2 = \frac{\partial\phi(a, b, c)}{\partial b} \quad \phi_3 = \frac{\partial\phi(a, b, c)}{\partial c}$$

Hoffmann & Vieira (1983) presented a procedure for the determination of the matrix of variances and covariances of the parameters and their estimates, based on the likelihood function.

## RESULTS AND DISCUSSION

Let us consider the equation  $W = (\alpha + \beta t) - (\alpha - w_0)\exp(-\gamma t)$ , where  $W$  is the seed water content at time  $t$ ;  $(\alpha + \beta t)$  is the asymptote of the exponential curve with  $\alpha$  equal to the asymptote value at time  $t = 0$  and  $\beta$  is its slope, representing the change in water absorption capacity during the seed imbibition process;  $w_0$  is the water content at time  $t = 0$  and  $\gamma$  is the constant rate related to seed permeability to water. Parameters  $\alpha$ ,  $\beta$  and  $\gamma$  can be estimated by methods found in the literature, such as the Marquardt - Levenberg method cited in Seber & Wild (1988), and will be represented by  $a$ ,  $b$  and  $c$  respectively. Thus,

$$\hat{W} = (a + bt) - (a - w_0)\exp(-ct)$$

The difference between the asymptote and the fitted model, represented by  $W^*$ , is given by

$$W^* = (a - w_0)\exp(-ct) \quad (2)$$

The point beyond which this difference is no longer significant is determined by Student's T statistics, at a selected significance level.

$$T = \frac{W^* - 0}{\sqrt{\hat{V}(W^*)}} \quad (3)$$

The variance estimate of the difference between the asymptote and the model can be determined by applying equation (1) into (2), and obtaining the expression:

$$\hat{V}(W^*) = [\exp(-ct)]^2 [\hat{V}(a) + t^2(a - w_0)^2 \hat{V}(c) - 2t(a - w_0) C\hat{\sigma}_v(a, c)] \quad (4)$$

Substituting in (3) the  $\hat{V}(W^*)$  expression given by (4), and the  $W^*$  expression, given by (2), we obtain

$$\left[ \frac{\hat{V}(a)}{(a - w_0)^2} T^2 - 1 \right] - \left[ \frac{2C\hat{v}(a,c)T^2}{(a - w_0)} \right] t + \hat{V}(c) T^2 t^2 = 0 \quad (5)$$

By solving this second-degree equation for t, we have

$$t = \frac{TC\hat{v}(a,c) \pm \sqrt{T^2(C\hat{v}(a,c))^2 - \hat{V}(c)\hat{V}(a)T^2 + \hat{V}(c)a^2 - 2\hat{V}(c)aw_0 + \hat{V}(c)w_0^2}}{\hat{V}(c)(a - w_0)T}$$

Calculating t involves determinations of the variance and covariance estimates of the parameter estimates involved in the expression of the difference between the asymptote and the fitted model. These estimates are obtained in the process of model fitting. Thus, let us consider the statistical model

$$W_i = (\alpha + \beta t_i) - (\alpha - w_0) \exp(-\gamma t_i) + u_i$$

and admit that a sample of n values for  $t_i$  and  $W_i$  is given. If the  $u_i$  values are independent random variables normally-distributed with mean equal to zero and variance  $\sigma^2$ , the likelihood function of the sample is:

$$L = \prod_{i=1}^n (2\pi\sigma^2)^{-\frac{1}{2}} \exp\left\{ -\frac{1}{2\sigma^2} (W_i - (\alpha + \beta t_i) + (\alpha - w_0) \exp(-\gamma t_i))^2 \right\}$$

The maximum likelihood estimates ( $a, b, c$  and  $s^2$ ) are the values of  $\alpha, \beta, \gamma$  and  $\sigma^2$  that maximize L, and therefore maximize  $\ln L$ .

The symmetrical information matrix  $D_{p \times p}$  has its elements defined by

$$D_{hi} = -E \left( \frac{\partial^2 \ln L}{\partial \alpha_h \partial \alpha_i} \right) \text{ for } h=1, \dots, p \text{ and } i=1, \dots, p$$

By applying the assumptions  $E(u_i) = 0$  and  $E(u_i^2) = \sigma^2$ , we obtain the matrix D

$$\begin{pmatrix} \frac{\sum(-1 + \exp(-\gamma t_i))^2}{\sum(-1 + \exp(-\gamma t_i)) t_i} & -\frac{\sum(-1 + \exp(-\gamma t_i)) t_i}{\sum t_i^2} & -\frac{(\alpha - w_0) \sum(-1 + \exp(-\gamma t_i)) t_i \exp(-\gamma t_i)}{(\alpha - w_0) \sum t_i^2 \exp(-\gamma t_i)} & 0 \\ -\frac{\sum(-1 + \exp(-\gamma t_i)) t_i}{\sum t_i^2} & \frac{\sum t_i^2}{\sum t_i^2} & \frac{(\alpha - w_0) \sum t_i^2 \exp(-\gamma t_i)}{(\alpha - w_0)^2 \sum t_i^2 \exp(-2\gamma t_i)} & 0 \\ -\frac{(\alpha - w_0) \sum(-1 + \exp(-\gamma t_i)) t_i \exp(-\gamma t_i)}{0} & \frac{(\alpha - w_0) \sum t_i^2 \exp(-\gamma t_i)}{0} & \frac{(\alpha - w_0)^2 \sum t_i^2 \exp(-2\gamma t_i)}{0} & 0 \end{pmatrix} \frac{1}{2\sigma^2}$$

The estimate of the matrix of asymptotic variances and covariances for  $a, b$  and  $c$  is:

$$\hat{V} = \begin{pmatrix} \frac{\sum(-1 + \exp(-c t_i))^2}{\sum(-1 + \exp(-c t_i)) t_i} & -\frac{\sum(-1 + \exp(-c t_i)) t_i}{\sum t_i^2} & -\frac{(\alpha - w_0) \sum(-1 + \exp(-c t_i)) t_i \exp(-c t_i)}{(\alpha - w_0) \sum t_i^2 \exp(-c t_i)} \\ -\frac{\sum(-1 + \exp(-c t_i)) t_i}{\sum t_i^2} & \frac{\sum t_i^2}{\sum t_i^2} & \frac{(\alpha - w_0) \sum t_i^2 \exp(-c t_i)}{(\alpha - w_0)^2 \sum t_i^2 \exp(-2c t_i)} \end{pmatrix}^{-1} S^2$$

where  $s^2$  is the residual mean square obtained in the analysis of variance for the model.

For the data generated with the model proposed by Studdert et al. (1994), we obtain  $\hat{W} = (0.434 + 0.00162 t) - (0.434 - w_0) \exp(-0.121 t)$ , with  $r^2 = 0.98$ ,  $W^* = (0.434 - w_0) \exp(-0.121 t)$  and

$$\hat{V} = \begin{pmatrix} 2.75E - 04 & -3.76E - 06 & -2.05E - 04 \\ & 5.64E - 08 & 2.68E - 06 \\ & & 2.60E - 04 \end{pmatrix}$$

Substituting the values in expression (5) we get

$$-0.9897 + 0.005251 t + 0.001144 t^2 = 0.$$

Therefore,  $t = 27.2$  hours with  $W^* = 1.28\%$ .

The t value found is smaller than the determined value (29.2 hours), considering as a criterion for the stage change the 1% difference between the asymptote and the value estimated by the fitted model, as established by Blacklow (1972). This 2-hour difference corresponds to 0.28% in absorbed water.

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