Effects of Different Temperatures on the Performance of Seeds Germination *of Cecropia pachystachya* Trec. (Cecropiaceae)

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ABSTRACT

Seed germination of *Cecropia pachystachya* Trec. was investigated at 25, 30, and 35^{0}C using fluorescent day lights. Ten replicates each of 200 seeds were used in each thermal treatment. Germitest® paper, CEL-065®, and 11x11x5 cm Gerbox® were also used. The frequencies of radicle protrusions were counted at consecutive two hour intervals. The cumulative frequencies were combined with the distribution of Weibull using the following model: $Y = M (1 - \exp(-(t/b)^c))$. The best performance was achieved with the temperature of 30^{0}C (M, 95.29%). Time to achieve 60.23% of seed germination (0.6321 x 95.29%) was 99.35 h with a spread of 7.19. All model/data set combinations had close to linear.

Key words: *Cecropia pachystachya*; seed germination; Weibull function

INTRODUCTION

Cecropia pachystachya is a pioneer species (Budowski, 1965) occurring from Ceará, Bahia, Minas Gerais, Goiás, and Mato Grosso do Sul to Santa Catarina (Lorenzi, 1992). It is nondeciduous, heliophyte and selective hygrophyte, characteristic of secondary woods. In addition to its value as an ornamental, it is used for recovering of degraded areas (Lorenzi, 1992). The fruits are produced annually in large quantities and are of great importance as food for animals. Although fundamental to knowledge of the species, basic information on factors affecting seed germination performance of *C. pachystachya* is limited and requires additional studies.

Laboratory evaluation methods, carried out under controlled conditions, have been studied and developed in order to obtain regular, rapid, and complete seed germination. For tropical and subtropical tree and shrub species, whose specific requirements are often unknown, no standardized methodology exists, although some research-based information can be found (Figliolia *et al*., 1993).

The main factor affecting the metabolic processes responsible for germination of forest seeds is the temperature, which is directly associated with the ecological characteristics of the species (Figliolia *et al*., 1993). Thus, the optimum temperature and the time interval during which germination occurs vary among species. Some require alternating temperatures to germinate, others show responses to a narrow or wide thermal range (Figliolia *et al*., 1993). Many factors may alter the responses obtained. The physiological condition of the seed (Popinigis, 1977), and the age and origin of the biological material (Lockley, 1980) are the examples. The temperature can also have influence on seedling establishment in tropical forests, and it is a fundamental variable for studies of ecophysiology and plant succession (Figliolia *et al*., 1993). Appropriate temperatures permit a seed lot to express its maximum germination potential, an aspect of seed quality.

The physiological quality of a seed lot is evaluated by the germination (Ista, 1993), and vigor tests (Popinigis, 1977; Vieira & Carvalho, 1994). The results expressed using percentage as

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well as indices have generated doubts as to its power to discriminate different samples (Brown & Mayer, 1986, 1988a). In spite of difficulties in quantifying seed germination performance, some mathematical models have been proposed to overcome it. Models of cumulative distribution with the logistic function (Bahler *et al*., 1989; Dumur *et al*., 1990), the cumulative normal distribution (Dumur *et al*., 1990), and other functions (Brown & Mayer, 1988b) have been compared with the standards used. The model which has the best performance was proposed by Weibull (Weibull, 1951) and studied by several investigators (Brown & Mayer, 1988b; Bahler *et al*., 1989; Dumur *et al*., 1990; Carneiro, 1994a).

The distribution of Weibull permits greater precision in evaluating quality, because the unique expression of percent germination is replaced by parameters with biological meaning (Dumur *et al*., 1990 Carneiro, 1994a;). Previous results have indicated that this distribution is appropriate for evaluation of the performance of seeds under germination (Carneiro, 1994b; Carneiro & Guedes, 1995; Takahashi *et al*., 1996).

The objective of the present investigation was to quantify the germination performance of seeds of *Cecropia pachystachya* using three temperatures, combining cumulative measurements of germination frequencies with the cumulative distribution of Weibull.

STUDY AREA

The seeds used in this experiment were obtained from mature fructescences of *Cecropia pachystachya* Trec., collected from several trees, in fragments of the Semideciduous Seasonal Alluvial and Submontane Forest (Campos & Souza, 1997). This forest is located on the Upper Paraná River floodplain in the vicinity of the Municipalities of Porto Rico in the State of Paraná, and Taquaruçu in the State of Mato Grosso do Sul (22^0 43'/22^0 48'S and 53^0 15'/53^0 20'W) (Fig. 1).

The regional climate is classified in the Köppen system as Cfa, with a warm summer and the mean temperature of the warmest month is above 22^0C. The annual mean temperature is 22^0C and annual precipitation is between 1200 to 1300 mm (Maack, 1981; Campos & Costa-Filho, 1994).

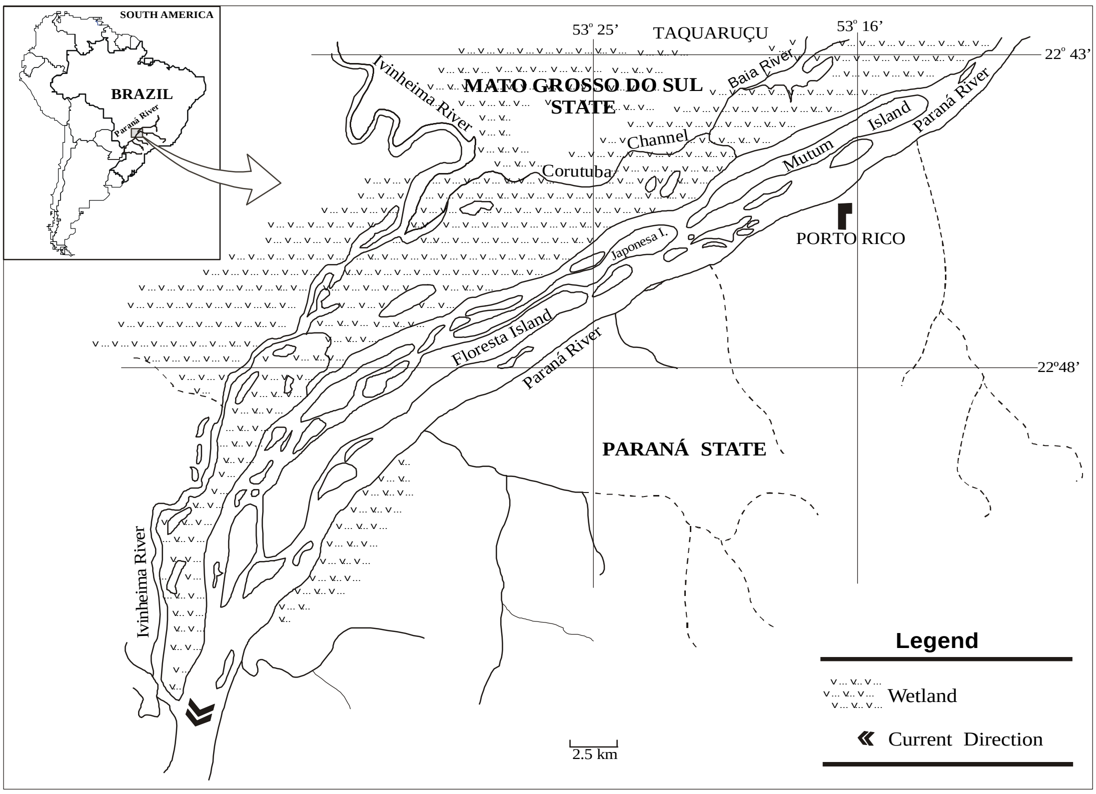

Fig. 1. Location of the study area

MATERIALS AND METHODS

The seeds were germinated at constant temperatures of 25, 30, and 35^{0}C, using germination chambers installed in a room with temperature at 20^{0}C ± 1^{0}C. "Daylight" fluorescent bulbs were kept turned on during the entire period of the experiment. Each treatment consisted of 10 repetitions, each of 200 seeds (Carneiro, 1994b; 1996), distributed uniformly on special germination paper, CEL-065®, in Gerbox® transparent plastic boxes measuring 11x11x5 cm. The substrate was kept humid with distilled deionized water. Germination frequencies were counted at consecutive time intervals of two hours (Carneiro, 1994b). Each seedling with the radicle lenght equal or greater than 3 mm were classed as germinated, counted and removed from the Gerbox.

Theoretical Foundation

The variable T is associated to the time until seed germination occurs. $F^*_T(t_i) = P(T > t_i)$, in that $P(T > t_i)$ indicates the probability that a seed, under test, will not germinate before time t_i, **i** being 1, 2, ..., and $F_T(t_i) = 1 - F^*_T(t_i)$ is the probability that a germination will occur. The expression:

Pg(t_i)=l i m <u>P(of a seed germinating in the time interval (t_i , t_i +) t)</u>
)t→ 0)t

describes germination power probabilistically, where (t_i , t_i +)t) is a finite and infinitesimal time interval (TSENG & HSU, 1989). Therefore, the power of seed germination is:

Pg (t_i) = l i m <u>1</u> P (t_i < T < t_i +)t | T > t_i)
)t→ 0) t
or,

Pg (t_i) = l i m <u>1</u> P <u>(t_i < T< t_i +)t | T > t_i)</u>
)t→ 0)t P (T > t_i)

The power of seed germination is related to the remaining seeds which have not yet germinated (Richards, 1959), and is expressed as:

$Pg(t_i) = (1/(1-F_T(t_i)))\ (dF/dt)$, in that dF/dt represents the differential of the cumulative function in relation to the time, indicating the instantaneous rate of variation, that is, $f_T(t_i)$ (Carneiro, 1994). Therefore,

$$Pg(t_i) = (1/(1-F_T(t_i)))\ (f_T(t_i)).$$

The cumulative distribution of Weibull (Weibull, 1951) is defined by the equation:

$$F_T(t_i) = 1 - \exp(-((t_i-t_0)/b)^c)$$

with the probability density function,

$$dF_T/dt = f_T(t_i) = [(c/b)(t_i-t_0)/b)^{(c-1)}]\ \exp(-(t_i-t_0)/b)^c$$

that is used to estimate the frequency at which seeds germinate in each time interval (Carneiro, 1994a). Thus, it is possible to describe the power of seed germination by the potential component of $f_T(t_i)$ in which $Pg(t_i) = (c/b)(t_i-t_0)/b)^{(c-1)}$. It indicates the conditional probability of the protrusion of the radicle occurring within a defined time interval (Carneiro, 1994a). The relative germination rate is obtained by the logarithm of the same component (Carneiro, 1994a; Takahashi *et al*., 1996).

Data Analysis

The data set with cumulative frequencies of seed germination were combined with the distribution of Weibull (Weibull, 1951) using the following parameterization: $Y = M(1 - \exp(-(t/b)^c))$ (Carneiro, 1994a). The additional parameter **M** indicates the maximum percentage of seed germination and is necessary due to the aspects related to the censored cases (Brown, 1987; Brown & Mayer, 1988b; Carneiro 1994a, 1996). The parameter **b** is the time to reach 63.21% of the maximum (M), and the **c** is a shape parameter which reflects skewness and accounts for the greater versatility of the distribution (Brown & Mayer, 1988b). The parameter t_0, estimates the lag phase (the time between the start of incubation and when the first seeds germinate) (Brown,1987), and can be removed from the model because its absence does not compromise the evaluation and contributes toward reducing nonlinearity (Carneiro, 1994).

The asymmetries of the parameters were estimated by the Hougaard coefficient (Hougaard, 1985). Values for g_1 lower than 0.1 indicate that the parameter estimate is close to linear. From 0.1 to 0.25, the estimate is reasonably close to linearity. Values greater than 0.25 indicate some asymmetry, and values greater than 1.0 are characteristic of totally nonlinear performance (Ratkowsky, 1990). The percentage of bias (Box, 1971) evaluated the stability of these estimates (Ratkowsky, 1990). The curvature of the solution locus in sample space was evaluated by the intrinsic nonlinearity, IN, and the lack of parallelism and inequality of spacing of the parameter lines on the tangent plane at the solution locus were evaluated by the parameter-effects nonlinearity, PE (Bates & Watts, 1980; Ratkowsky, 1986). The locus represents all possible solutions in the sample space. (Ratkowsky, 1986). The divergence between the tangent plane and the sampling space was evaluated by F test at the 5% probability level (Bates & Watts, 1980; Ratkowsky, 1990).

BMDP®, STATISTIC® and the subroutines of Ratkowsky (1990) were used to estimate the parameters and for calculation the power, probability, and relative rate of seed germination.

The discrimination among the treatments was evaluated by comparing the sums of squares of the residuals obtained for each treatment, with the sum of squares for the set of all observations (Mead *et al*., 1996).

RESULTS AND DISCUSSION

The results from combining germination frequencies with the cumulative distribution of Weibull are shown in Table 1. The best performance of the seeds under germination was achieved with the temperature of 30^0C. The maximum percentage (M) was 95.29%, and the time (b) to achieve 60.23% (0.6321 x 95.29%) was 99.35 h. Under temperatures of 25^0C and 35^0C, the estimates of M reached reasonably high values, but the time to b were 124.3 h and 127.27 h, respectively. The value of the spread (c) at 30^0C was 7.19, indicating that the distribution was close to the peaked normal with a small standard deviation (Dodson, 1984).

The Hougaard coefficient indicates estimates of asymmetry lower than 0.1 and the percent bias less than 1%. At the three temperatures, these parameters had a performance close to linear. (Ratkowsky, 1990). The intrinsic nonlinearity, IN, and those due to parameterization, PE, were not-significant ($P \leq 0.05$).

Table 1. Evaluation of the germination performance of seeds *Cecropia pachystachya* Trec. at three temperatures using the parameters of the Weibull cumulative distribution function.

Temperatures	Parameters		Hougaard's Coefficient	Percentage of bias	IN	PE
25^oC	M	92.82	0.000039	0.0006	0.034 NS	0.0438 NS
	b	124.3	0.014073	0.0003		
	c	10.31	0.044516	0.0108		
30^oC	M	95.29	0.002979	0.0023	0.0588NS	0.0617 NS
	b	99.35	0.019501	0.0009		
	c	7.19	0.071130	0.0259		
35^oC	M	89.66	0.015978	0.0042	0.0496 NS	0.0447 NS
	b	127.27	0.019106	0.0018		
	c	4.89	0.048180	0.0057		

M = Maximum percentage of seed germination; b = Time until 63,21% of M to occurs; c = The shape parameter indicating the spread of seed germination in time; IN = Intrinsic Nonlinearity; PE = Parameter-effects Nonlinearity; NS = not-significant.

Comparison among treatments, using the residual variance (Table 2) indicated that the performance of seed germination was different at each temperature. Although the differences in the percentages of seed germination were not large, there were differences in performance, with decrease and increase in the time necessary to reach 63.21% M, and in the estimates of c.

Table 2. Comparisons of the residual sums of squares of the models adjusted for each separate and combined data set.

Source of variation	RSS	d.f	Ms	F
25^{o} C	11623.0	620		
30^{o} C	31311.3	580		
35^{o} C	37084.3	740		
∑ residual	80018.6	1940	41.246	
Residual for combined data	279884	1946		
Difference between fitted models	199865.4	6	33310.9	807.61

The probabilities of seed germination are shown in Fig. 2. It could be seen that at 30^{0}C, the time to reach 99% of maximum (M) was 130 h. At 25^{0}C and 35^{0}C, this value occurred only after 150 and 192 h respectively.

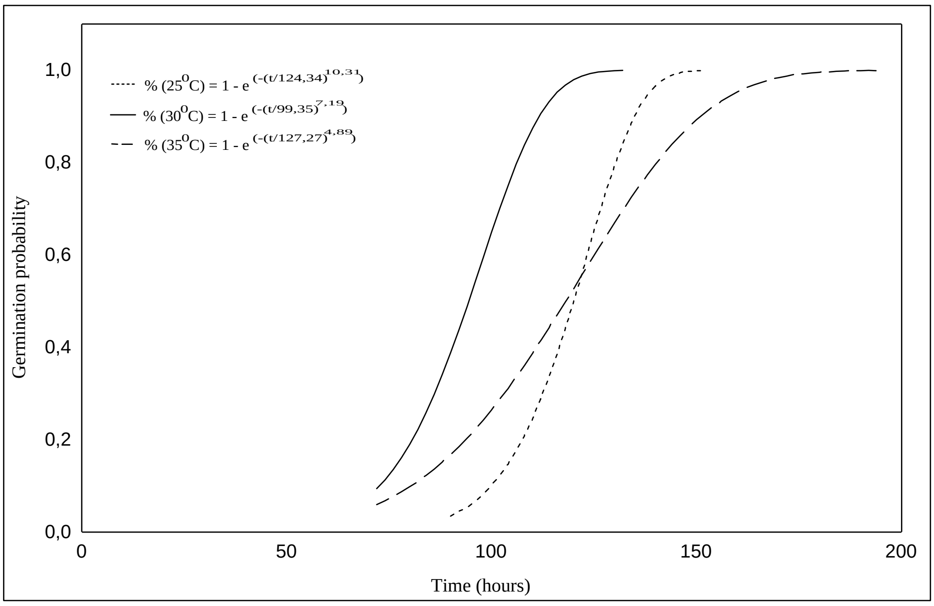

Fig. 2. Probability curves for germination of seeds of *Cecropia pachystachya* at three temperature

The power of seed germination are presented in Fig. 3. It was found that the seeds which germinated at 30^{0}C had a higher germination power than those germinated at 25^{0}C and 35^{0}C. At 30^{0}C, the seeds germinated over a shorter time interval. Once germination had begun, it proceeded rapidly (Fig. 4). Thus, at this temperature, the chance of germination of a new seed cohort increased in each new period of occurrence of this event, i.e., the cumulative chance of germination ocurrences was high.

The start of seed germination at 25^{0}C was delayed, compared to 30^{0}C and 35^{0}C. At 25^{0}C, radicle protrusions began after 90 h from the beginning of the test, while at 30^{0}C and 35^{0}C, initial protrusions occurred after 72 h. Nevertheless, once begun, the process was quite rapid, since at 25^{0}C the protrusions terminated before that at 35^{0}C. This could be seen in the steeper slope of the logarithmic curve (Fig. 4). At 35^{0}C, the relative rate of seeds germination declined, as indicated by the lower slope of the straight line (Fig 4). This slower and more dispersed germination over time was due to the lower value of the parameter c associated with the value of b. According to Richards (1959), values of c near 3 indicated a deceleration of the phenomenon evaluated.

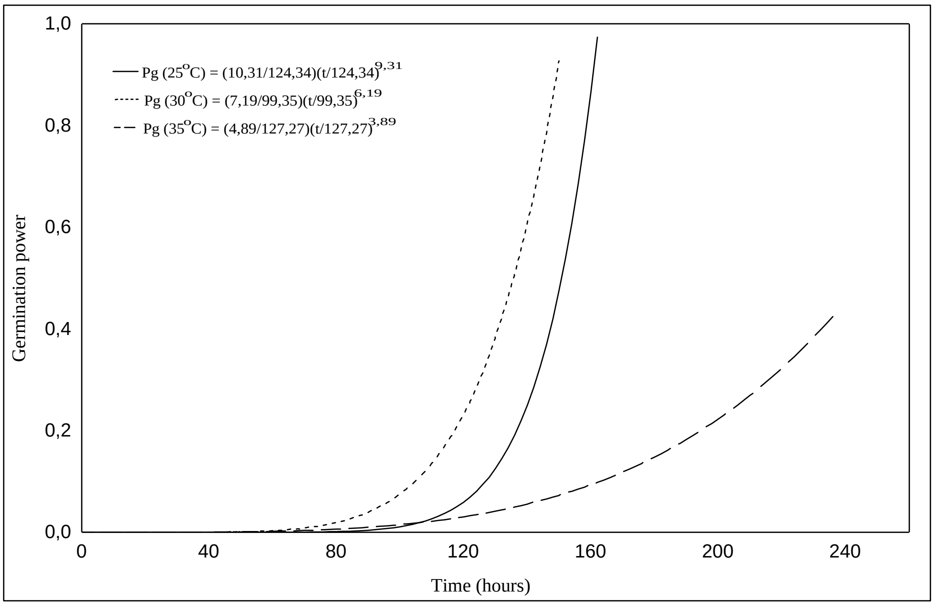

Fig. 3. Curves for the power of seeds germination of *Cecropia pachystachya*, calculated from estimates of the parameters of the cumulative distribution of Weibull

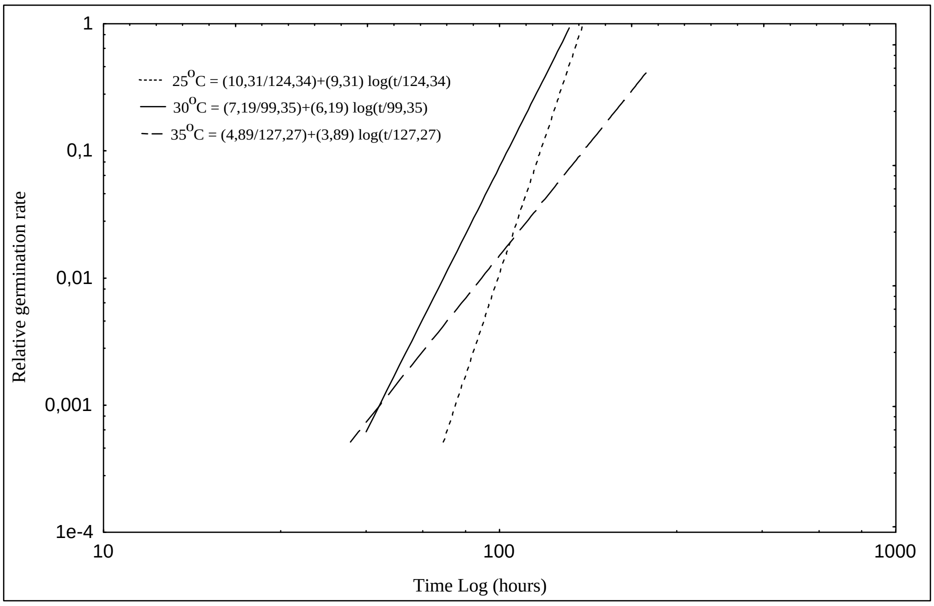

Fig. 4. Relative rate of seeds germination of *Cecropia pachystachya*, calculated from estimates of the parameters of the cumulative distribution of Weibull

The best performance of seeds obtained at 30^0C, is in agreement with investigators who suggested the thermal interval between 20^0C and 30^0C as adequate for seed germination of a large number of tropical and subtropical species (Whitmore, 1982; Borges & Rena, 1993). Nevertheless, species with widely distributed populations, such as *C. pachystachya*, may show variability in the germination responses according to their geographical distribution (Yousheng & Sziklai, 1995). Seeds of *C. pachystachya* collected in the region of Moji Guaçú, State of São Paulo, for example, when set out to germinate in beds of sand and in the open air, had only 34% of germination (Barbosa *et al*., 1997), a value lower than the results found in present studies. Evaluation of seeds of *C. obtusifolia* at Vera Cruz, Mexico presented 80% germination at 26^0C and a strong reduction to 18% when a temperature of 36^0C was used (Vazquez-Yanes, 1976). These results do not match with on findings, but are in agreement with the decrease in percentage of seed germination at temperatures greater than 30^0C. However, other studies at Vera Cruz, Mexico, using the same species, *C. obtusifolia*, reported germination percentages higher than 90% at 25^0C (Alvarez-Buylla & Martinez-Ramos, 1990; AlvarezL-Buylla & Garay, 1994).

The estimates of seed germination obtained here corroborate the observation of Carneiro (1994b), who showed that evaluation of germination of seeds under favourable conditions, and with measurements taken over short time intervals, favour the combination model/data set.

CONCLUSIONS

The results obtained in this experiment recommend the temperature of 30^0C for the standard germination test for seeds of *Cecropia pachystachya*. The temperature of 25^0C, although delayed the time the first radicles protrusions, provided a higher relative rate of seed germination.

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RESUMO

Com o objetivo de avaliar o desempenho germinativo de sementes de *Cecropia pachystachya* Trec., sob diferentes níveis de temperatura, foi realizado um experimento com sementes coletadas em fragmentos da Floresta Estacional Semidecidual Submontana e Aluvial, localizados na planície de inundação do alto rio Paraná. As sementes foram submetidas a temperaturas constantes de 25^0C, 30^0C e 35^0C. Luzes fluorescentes do tipo “luz do dia” foram mantidas acesas durante todo o período experimental. Cada tratamento consistiu de 10 repetições de 200 sementes distribuídas de maneira uniforme sobre papel especial de germinação, CEL-065, em caixas de plástico transparente do tipo gerbox medindo 11x11x5 cm. As freqüências germinativas foram avaliadas em intervalos consecutivos de duas horas. As freqüências acumuladas foram combinadas com o seguinte modelo da função de Weibull: $Y = M (1- \exp (-(t/b)^c))$. Os resultados indicaram que o melhor desempenho germinativo foi alcançado com a temperatura de 30^0C. A porcentagem máxima de germinação (M) foi igual a 95,29%, e o tempo (b) para a ocorrência de uma porcentagem de germinação igual a 60,23% (0,6321 x 95,29%) foi igual a 99,35 horas. A dispersão das germinações (c) durante o tempo de avaliação foi igual a 7,19, indicando que a distribuição não se afastou muito da normal com pequeno desvio padrão. Ficou evidenciado também, que as estimativas dos parâmetros tiveram um desempenho próximo à linearidade.

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