

Germination response of *Hylocereus setaceus* (Salm-Dyck ex DC.) Ralf Bauer (Cactaceae) seeds to temperature and reduced water potentials

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(With 4 figures)

Abstract

The germination response of *Hylocereus setaceus* seeds to isothermic incubation at different water potentials was analysed by using the thermal time and hydrotime models, aiming to describe some germination parameters of the population and to test the validity of the models to describe the response of the seeds to temperature and water potential. *Hylocereus setaceus* seeds germinated relatively well in a wide range of temperatures and the germination was rate limited from 11 to 20 °C interval and beyond 30 °C until 40 °C, in which the germination rate respectively shifts positively and negatively with temperature. The minimum or base temperature (T_b) for the germination of *H. setaceus* was 7 °C, and the ceiling temperature varied nearly from 43.5 to 59 °C depending on the percent fraction, with median set on 49.8 °C. The number of degrees day necessary for 50% of the seeds to germinate in the infra-optimum temperature range was 39.3 °C day, whereas at the supra-optimum interval the value of $\theta = 77$ was assumed to be constant throughout. Germination was sensitive to decreasing values of Ψ in the medium, and both the germinability and the germination rate shift negatively with the reduction of Ψ , but the rate of reduction changed with temperature. The values of base water potential (Ψ_b) shift to zero with increasing temperatures and such variation reflects in the relatively greater effect of low Ψ on germination in supra optimum range of T. In general, the model described better the germination time courses at lower than at higher water potentials. The analysis also suggest that T_b may not be independent of Ψ and that $\Psi_{b(g)}$ may change as a function of temperature at the infra-optimum temperature range.

Keywords: Cactaceae, *Hylocereus*, hydrotime, thermal time, water potential.

Resposta de germinação das sementes de *Hylocereus setaceus* (Salm-Dyck ex DC.) Ralf Bauer (Cactaceae) à temperatura e à redução do potencial de água

Resumo

As respostas de germinação de sementes de *Hylocereus setaceus* para incubações isotérmicas sob diferentes potenciais de água foram analisadas utilizando-se modelos de graus dia e psi dia, com o objetivo de descrever alguns parâmetros da população e para testar a viabilidade do modelo para descrever as respostas da semente a temperatura e potencial de água. Sementes de *H. setaceus* germinaram relativamente bem em uma ampla faixa de temperaturas e a germinação foi limitada pela velocidade nos intervalos de 11 a 20 °C, e de 30 até 40 °C, nos quais a velocidade de germinação aumenta e diminui, respectivamente, com a temperatura. A temperatura mínima ou base (T_b) para germinação de *H. setaceus* foi 7 °C, e a temperatura máxima variou de 43,5 a 59 °C dependendo da fração percentual, com média de 49,8 °C. O número de graus dia necessário para 50% das sementes germinarem na faixa de temperatura infra-ótima foi de 39,3 °C dia, enquanto que, no intervalo supra-ótimo o valor de 77 °C dia foi assumido como constante para todo o intervalo. A germinação foi sensível à diminuição do Ψ no meio, e tanto a germinabilidade como a velocidade de germinação mudaram negativamente com a redução do Ψ , sendo que a taxa de redução mudou com a temperatura. Os potenciais base de água ($\Psi_{b(g)}$) tendem a zero com o aumento da temperatura e essa variação se reflete no efeito relativamente maior do Ψ sobre a germinação na faixa supra-ótima de temperatura. No geral, o modelo descreveu melhor as curvas de germinação em potenciais de água baixos do que em altos. As análises também sugerem que T_b pode não depender do Ψ e que o $\Psi_{b(g)}$ pode mudar em função da temperatura na faixa infra-ótima de temperatura.

Palavras-chave: Cactaceae, *Hylocereus*, psi dia, graus dia, potencial da água.

1. Introduction

Successful establishment of plant species in a natural environment can be related to timing for germination resulting from seed response to environmental factors such as water availability, light and temperature. In *Medicago sativa* L. (alfafa), for example, rapid germination and primary root growth at infra-optimal temperatures in the laboratory was positively correlated with high emergence at field conditions (Klos and Brummer, 2000). Thus, germination cueing determines a particular set of environmental conditions that a germinant will encounter (Donohue, 2005) and seeds often respond to combinations of environmental factors that are most favourable to the seedling growth and survival (Simão et al., 2007; De La Barrera and Nobel, 2003)

Temperature is a very important factor controlling seed germination, affecting both the rate and final percentage of germination. Studies on temperature-dependent seed germination have taken into account cardinal temperatures, that is, the base or minimum temperature (T_b) below which seeds will not germinate, an optimal temperature (T_o) at which the best germination is attained, and a maximum or ceiling temperature (T_c) above which no germination occurs (Labouriau, 1983). Within a given range, only the germination rate (the reciprocal of the time taken for the germination) is affected by temperature and such germination rate dependency on temperature may be one of the most important factors controlling the emergence timing in the field, thus influencing the probability of seedling establishment (Washitani, 1985). Seeds do not respond simultaneously to temperature but germination is rather spread over time due to intrinsic characteristics of each seed. A valuable tool for analysing the spread of the germination rate was proposed by Garcia-Huidobro et al. (1982) based on the assumption that the order in which seeds germinate is independent of temperature, and each seed can be assigned a value of g_g (a germination fraction at which it germinates) that can be used to identify each seed or small group of seeds sharing an identical g_g or germination rate (reciprocal of the time to germination of the fraction g_g) (Gummerson, 1986). Thus, all the seeds of each fraction are assumed to germinate at the same time, and the fractions can be discriminated from each other by particular cumulative percentages at which they germinate (Washitani, 1985). The application of the model is based on the observation that there is a temperature range over which the germination rate for a given fraction g_g increases linearly with the temperature according to the equation: $1/t_g = (T - T_b)/\theta_g$, where t_g is the time to germination of the fraction g_g , T is the actual temperature and θ_g is the "thermal time", which is followed by the notation g to indicate that it varies from seed to seed. If all the seeds in a population germinated at the same time, the germination time course would be a step function and the time to germination of different fractions would be constant. The variation in the time to germination accounts for the

sigmoid shape of the germination time curve and must be caused by the seeds having different thermal time requirements (Gummerson, 1986). At supra-optimal temperatures, however, the germination rate decreases with temperature and it is the T_c that varies among fractions while the thermal time to radicle protrusion is a constant for all seeds. Thus, in the supra-optimal temperature range the following thermal time equation must be used (Garcia-Huidobro et al., 1982): $1/Tc_{(g)} = (T - T_c)/\theta$. For seeds, the thermal time represents the progress toward germination and it can give a good estimate of the time to emergence for specific crops.

Regardless of the temperature, germination can be delayed or prevented if there is insufficient water within the seed to generate the turgescence pressure required for cell expansion, thus seed germination is highly dependent on the water potential (Ψ) in the substrate (Bradford and Still, 2004). Gummerson (1986) proposed that seed germination responses to Ψ could be described on a "hydrotime" scale similar to the thermal time one, and many authors have analysed the effect of Ψ on seed germination in a manner analogous to that. Gummerson (1986) defined a hydrotime constant as $\theta_H = (\Psi - \Psi_{b(g)})t_g$, where $\Psi_{b(g)}$ is the threshold or base Ψ that will just prevent germination of fraction g . Considering θ_H as constant, the time required for a percentage g to germinate is inversely proportional to the difference between the medium Ψ and the Ψ_b of the respective fraction g . Within a seed population, the normal distribution of Ψ_b values among seeds accounts for the variation in θ_g among different seed fractions. In other words, the distribution of $\Psi_{b(g)}$ values results in a characteristic sigmoid cumulative time course of germination events (Bradford, 1997). Thus, the thermal and hydrotime models can also be combined to result in a hydrothermal time model according to the equation: $\theta_{HT} = (T - T_b)(\Psi - \Psi_{b(g)})t_g$, where θ_{HT} is the hydrothermal time constant (MPa °C time). This equation is useful to describe germination time courses at any combination of sub-optimal temperature and Ψ (Bradford, 1995), whereas a modified hydrothermal time model was proposed by Alvarado and Bradford (2002) to describe the germination timing and percentages across temperatures and Ψ at the supra-optimum temperature range.

Hylocereus setaceus (Salm-Dyck ex DC.) Ralf Bauer, commonly known as dragon fruit, strawberry pear and pitaya, is an epiphytic cactus, stems usually climbing on trees, sometimes clambering over rocks or walls, developing many aerial roots. Widespread in eastern Brazil from northern Piauí southwards; northern, central-western and southern Brazil (southern Pará southwards to Mato Grosso do Sul and Paraná); and South America (southwards to east Bolivia, north Argentina and Paraguay) (Britton and Rose, 1937). Seeds of *H. setaceus* are highly sensitive to light and germinate in a relatively wide range of temperature (Simão et al., 2007). Considering that the thermal and hydrotime approach can be used for expressing the relationship between temperature, water

potential and germination, and considering the gap in the scientific literature on thermal time analysis of germination of Cactaceae seeds, the aim of this study was to describe some germination parameters of *Hylocereus setaceus* seeds and to test the validity of the hydrotime model describing the seed germination in response to a range of water potentials and temperatures.

2. Material And Methods

Fruits of *H. setaceus* were harvested in December 2004 from three plants, in a fragment of seasonal semi-deciduous forest located at São Tomás de Aquino, MG, Brazil. The fruits were depulped on a sieve and the seeds were washed under tap water. For the de-pulping process, the pH of water was decreased with small doses of commercial vinegar (BELMONT®) and immediately washed with current tap water to remove the pulp firmly adhered to the seeds. After that, the seeds were dried at room temperature (around 25 °C) up to five days and stored at 10 °C in a sealed glass bottle until required (October 2006). The mass of 1000 seeds of the *H. setaceus* batch used in the experiments was 2.80 ± 0.0424 mg (Simão et al., 2007).

The assays were carried out in the Plant Photomorphogenesis Laboratory at the Botany Department, IB, Rio Claro, SP, Brazil. The seeds were sown on two layers of filter paper saturated with distilled water or solution, in 50 mm inner diameter glass Petri dishes, which were put inside colorless plastic boxes (Gerbox). Three replicates per treatment with 40 seeds each were placed in growth cabinets under continuous white light – two 20 W fluorescent tubes, $32.85 \mu\text{mol}\cdot\text{m}^{-2}/\text{s}$ at seed level – at the constant temperatures of 11, 14, 17, 20, 25, 30, 34, 37 and 40 °C (± 1 °C). In order to control the water potential of the germination medium, different solutions of polyethylene-glycol 6000 (PEG 6000) were prepared according to Vilella et al. (1991) that maintained the nominal water potentials of -0.1 , -0.2 and -0.35 MPa at the respective temperature. In order to maintain an approximate known Ψ the seeds were transferred to fresh PEG solutions as soon as the blotters appeared to be drying. The germination (radicle protrusion) was scored daily and the germinated seeds were removed.

The germinability (maximum germination) was the accumulated germination percentage recorded up to 30 days from the beginning of the assay. The germination rate was computed as the reciprocal of the time (days) to radicle emergence of an arbitrary percentage or fraction g ($g = 5, 10, 20, 30, 40, 50, 60, 70$ and 80%), estimated from the actual germination time courses fitted by the Weibull function (Dumur et al., 1990). The average germination rate (\bar{A}) was calculated according to the equation $\bar{A} = \sum_i n_i / \sum_i n_i \cdot t_i$, where n_i is the number of germinated seeds at the time interval t_i (Labouriau and Agudo, 1987).

The distributions of the cumulative germination percentages were analysed and the parameters were determined for the thermal time, hydrotime and hydrothermal time models. To find the values of base temperature (T_b), median thermal time (θ_{50}) and standard deviation (σ_θ) of the thermal time distribution, the cumulative daily germination percentages of *Hylocereus setaceus* seeds kept in distilled water at three temperatures (11, 14 and 17 °C) in the infra-optimum range were transformed to probit and plotted against the respective θ_g ($= [T - T_b]t_g$) on a logarithmic scale (Bradford, 1995). The data were combined into a common linear regression to determine T_b according to the model $\text{probit}(g) = \{\log[(T - T_b)t_g] - \log \theta_{50}\} / \sigma_\theta$, and repeated regression analyses were performed varying the value of T_b until the best fit (higher R^2) was obtained (Bradford, 1995). Then, θ_{50} is the θ value (x axis) necessary for 50% of the seeds to germinate ($=5$, on the probit axis), and σ_θ is the inverse of the slope of the probit regression line. A similar procedure was used to determine the median ceiling temperatures (T_{c50}), σ_{Tc} and θ , substituting $[(T - T_b)t_g]$ for $[T + \theta/t_g]$ and θ_{50} for T_{c50} in the model above, with temperatures at the supra-optimum range (34, 37 and 40 °C). Thus, $\text{probit}(g)$ data were regressed on $\log T_c$ ($T_c = T + \theta/t_g$) and different values of θ were tried until the best fit. The median T_c is the T_c value (x axis) corresponding to the fraction 50% (probit = 5 on the y axis), and σ_{Tc} is the inverse of the slope.

The repeated probit analysis technique referred to above was used to estimate the values of θ_H , median ψ_b ($= \Psi_{b(50)}$) and $\sigma_{\psi b}$ (the standard deviation of ψ_b among seeds). For the infra-optimum temperatures (11, 14 and 17 °C) the linear model was $\text{probit}(g) = [\Psi - (\theta_H/t_g) - \Psi_{b(50)}] / \sigma_{\psi b}$ (Bradford, 1995). Daily cumulative germination percentages at -0.1 , -0.2 and -0.35 MPa were combined into a single regression of $\text{probit}(g)$ (y axis) as a function of $\psi_{b(g)}$ ($= \Psi - \theta_H/t_g$), and different values of θ_H were used until the highest R^2 was obtained (Bradford, 1995). Median ψ_b is then the midpoint of the regression line ($\text{probit}(g) = 5$), and $\sigma_{\psi b}$ is the reciprocal of the slope. One regression was performed for each infra-optimum temperature and the $\psi_{b(g)}$ distributions were used to generate theoretical germination time courses at each infra-optimal T and ψ combination (Alvarado and Bradford, 2002). Both temperature and ψ responses of germination at the infra-optimum temperature range can also be combined and expressed on a hydrothermal time basis, according to the model $\text{probit}(g) = \{[\Psi - \theta_{HT} / ((T - T_b)t_g) - \Psi_{b(50)}] / \sigma_{\psi b}\}$ where θ_{HT} is a hydrothermal time constant (Bradford, 1995). The parameters θ_{HT} , $\Psi_{b(50)}$ and $\sigma_{\psi b}$ were found by combining the germination time courses at different infra-optimal temperatures and ψ into a single regression of $\text{probit}(g)$ on $[\Psi - \theta_{HT} / ((T - T_b)t_g)]$. Different values of θ_{HT} were entered until the best fit, and $\Psi_{b(50)}$ and $\sigma_{\psi b}$ were estimated as above.

For the supra-optimum temperature range the hydrothermal time model was $\text{probit}(g) = \{[\Psi - k_1(T - T_o)] - (\theta_H/t_g) - \Psi_{b(50)}\} / \sigma_{\psi b}$, where k_1 is the slope of the $\psi_{b(g)}$ versus T line when $T > T_o$ (optimal temperature) and θ_H is the

hydrotime value at T_o . Germination time courses of seeds incubated in -0.1 , -0.2 and -0.35 MPa at the temperatures of 34 , 37 and 40 °C were combined into a single regression of $\text{probit}(g)$ on $[\Psi - k_T(T - T_o) - \theta_H/t_g]$. Following Alvarado and Bradford (2002), this model was fitted by changing systematically T_o , k_T and θ_H until the median ψ_b for this model was equal to the $\psi_{b(s0)}$ of the hydrothermal time model at the infra-optimum temperature range. Note that this model stops the accumulation of thermal time at the value equivalent to that accumulated at T_o . Thus, temperatures above T_o do not add thermal time in the supra-optimal range; and effects on germination are accounted for by the change in $\psi_{b(g)}$ (Bradford, 1995). The hydrotime parameters at the temperatures of 34 , 37 and 40 °C were derived by fitting the combined ψ data at each temperature using the common θ_H obtained through the supra-optimal hydrothermal time model (Alvarado and Bradford, 2002). The $\psi_{b(g)}$ distributions obtained at each supra-optimal temperature were then used to produce the predicted germination curves at the different ψ and supra-optimal T .

3. Results

In order to find the cardinal temperatures of the seed batch, *Hylocereus setaceus* seeds were germinated in distilled water under white light at different constant temperatures. The germinability (final germination) in the temperature range of 11 to 40 °C was relatively high in practically all the isotherms. With the exception of 40 °C, in which the germinability was around 70% , the final germination at the different temperatures attained values between 80 and 100% (Figure 1a). The relative homogeneity of the seed population with regard to germination capacity allowed the germination rate indexes at the different temperatures to be compared to each other. The average germination rate increased with temperature at the range of 11 to 20 °C, and decreased above 30 °C. The germination rate did not vary in the 20 to 30 °C range (Figure 1b). Thus, the effect of the PEG solutions was tested at temperatures of 11 , 14 and 17 °C, considered as infra-optimal, and at 34 , 37 and 40 °C, considered as supra-optimal.

The distributions of accumulated germination percentages of *Hylocereus setaceus* seeds incubated in different PEG 6000 solutions show that increasing PEG concentrations (= reducing water potentials) can affect both the beginning of the germination and the germinability (Figure 2 and Table 1). At infra-optimal temperatures, germination is delayed at lower nominal water potentials, particularly at -0.2 and -0.35 MPa. As compared to the distilled water control, germinability decreased significantly only for the seeds incubated in the -0.35 MPa solution at the temperatures of 14 and 17 °C (Table 1). When the seeds were imbibed in reduced ψ at the supra-optimum temperature range, a delay in germination was observed relative to the seeds kept in distilled water (Figure 2), and also the final germination was inhibited

in seeds incubated in -0.35 MPa solutions at 34 , 37 and 40 °C, as well as in -0.2 MPa solution at 37 °C (Table 1). The solid lines in the Figure 2 are based upon the $\psi_{b(g)}$ threshold distributions predicted by the hydrotime model. In general, the lines described relatively well the distributions of the observed cumulative germinations percentage in the 0 and -0.1 MPa treatments, whereas the predicted values show a poor agreement with the observed ones at the nominal water potentials of -0.2 and -0.35 MPa.

Figure 3 shows the relationship between germination rate (the reciprocal of the time to a given fraction to germinate) and ψ . In order to standardise the y scales some fractions (symbols in Figure 3) were omitted for seeds germinated at 17 °C in distilled water ($\psi = 0$ MPa). In general, the germination rate decreased with the reduction of the ψ , but the effect depended on the temperature. At the infra-optimum range the slope of the regression line of the germination rate on ψ increases with temperature, whereas at the supra-optimal interval the slope reduces with increasing temperatures (Figure 3). At 40 °C there

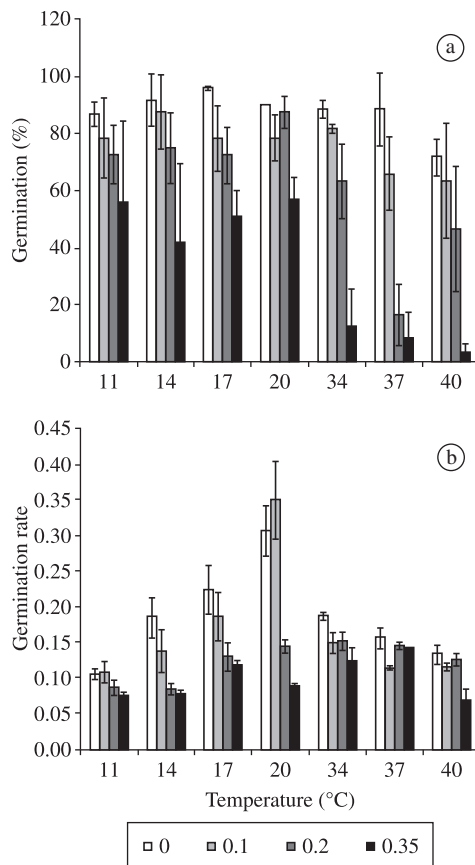


Figure 1. Dependence of temperature on germinability (a) and germination rate (b) of *Hylocereus setaceus* seeds incubated at 0 , 0.1 , 0.2 and 0.35 MPa. Data after 30 days of incubation. Germination rate taken as the reciprocal of the average germination time (days). Confidence intervals ($\alpha = 0.05$) are presented.

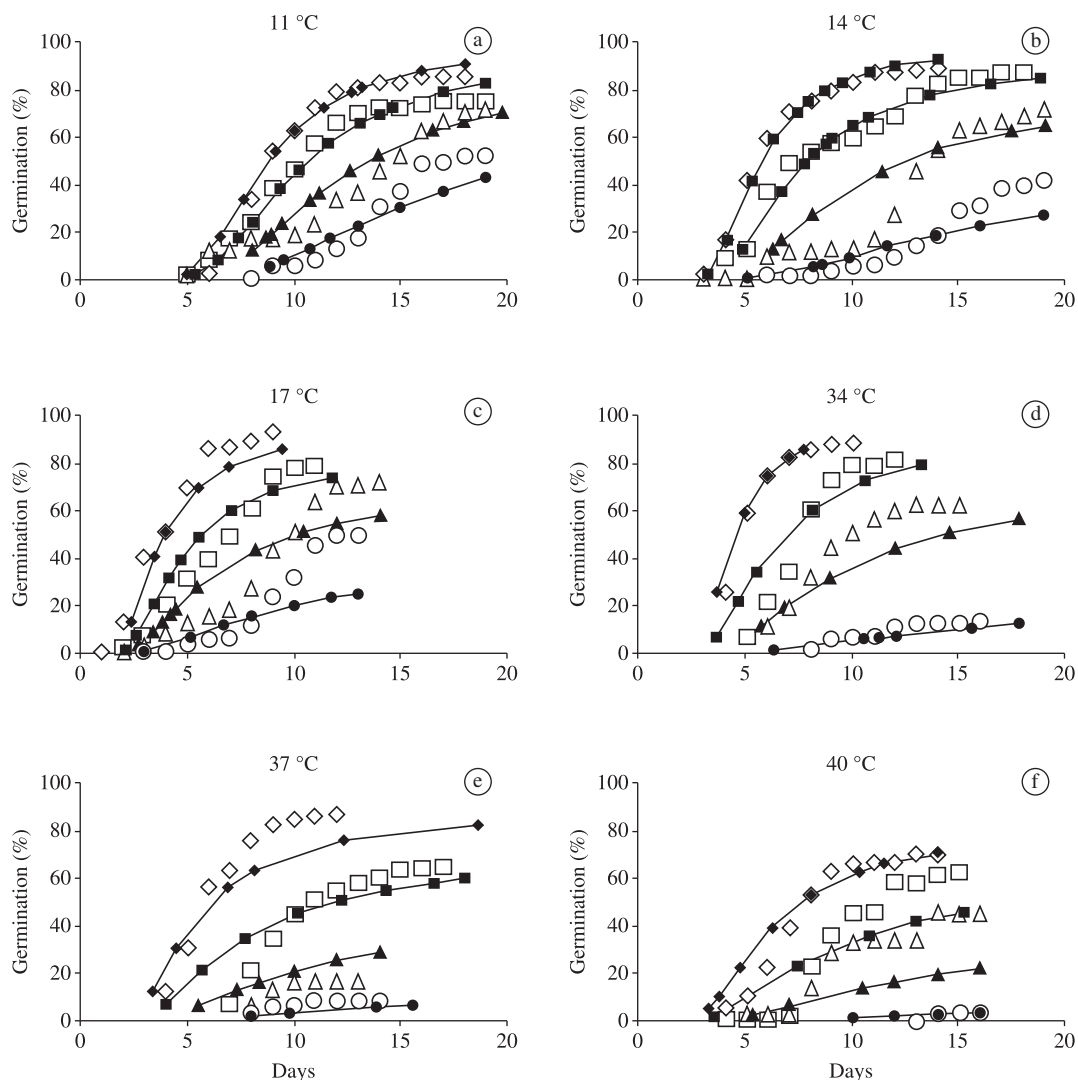


Figure 2. Cumulative distribution of germination percentages of *Hylocereus setaceus* seeds incubated in PEG solutions with osmotic potentials of 0 MPa (distilled water control) (\diamond), -0.1 MPa (\square), -0.2 MPa (\triangle) and -0.35 MPa (\circ) at different constant temperatures. Symbols are the actual data and lines are the predicted values by the model $\text{probit}_{(g)} = [\psi - (\theta_H/t_g) - \psi_{b(50)}] / \sigma_{\psi_b}$. Parameter values are displayed in Table 2.

Table 1. Statistical comparisons amongst water potentials (Ψ) and temperature treatments on the germinability of *Hylocereus setaceus* seeds. Letters (LSD_{Tukey}, $\alpha = 0.05$, $n = 3$) compare germinability (final germination); small letters = horizontal comparisons; capital letters = vertical comparisons.

Ψ (MPa)	Temperature (°C)					
	11	14	17	34	37	40
0.0	86.7Aa	91.7Aa	95.8Aa	88.3Aa	89.1Aa	71.7Aa
-0.1	78.3Aa	87.5Aa	78.3ABa	81.7Aa	65.8Aa	63.3Aa
-0.2	72.5Aa	75.0ABa	72.5ABa	63.3Aa	16.7Bb	46.7Aab
-0.35	55.8Aa	41.7Bac	50.8Bab	12.5Bb	8.33Bc	3.33Bc

was no relationship between the germination rate and ψ since the determination coefficient was not significant.

Assuming that the germination rate of *Hylocereus setaceus* seeds changes linearly with temperature both

at the infra and supra-optimum range, and that T_b is relatively constant and independent of the water potential, the data were analysed according to the thermal time, hydrotime and hydrothermal time models. Firstly, the accumulated daily germination percentages of seeds im-

bibed in distilled water at 11, 14 and 17 °C were transformed to probit and regressed on $\log \theta_g [= (T - T_b)t_g]$, and the value of T_b that produced the best fit was 7 °C, taken as the minimum temperature for the germination of *H. setaceus* seeds. In the supra-optimum temperature range the values of $\text{probit}(g)$ were related to $\log T_{c(g)} [= T + \theta/t_g]$, and the best fit was obtained with $\theta = 77^\circ$ day. The number of degrees day necessary for 50% of the seeds to germinate in the infra-optimum temperature range was 39.3° day (Table 2), derive from the regression line of $\text{probit}(g)$ on $\log \theta_g$. In the supra-optimum

interval the value of $\theta = 77$ was assumed to be constant throughout, and the limiting factor for germination would be the distribution of $T_{c(g)}$ within the population. The values of $T_{c(g)}$ derived from the relationship between $\text{probit}(g)$ at 34, 37 and 40 °C and T_c varied from nearly 43.5 to 59 °C, and the median T_c ($T_{c(50)}$) corresponding to the fraction 50% was 49.8 °C (Table 2).

The probit transformed germination percentages of *H. setaceus* seeds incubated in different ψ at each infra-optimum temperature were regressed on $\Psi_{b(g)} (= \psi - [\theta_H/t_g])$ and the values of θ_H that produced the

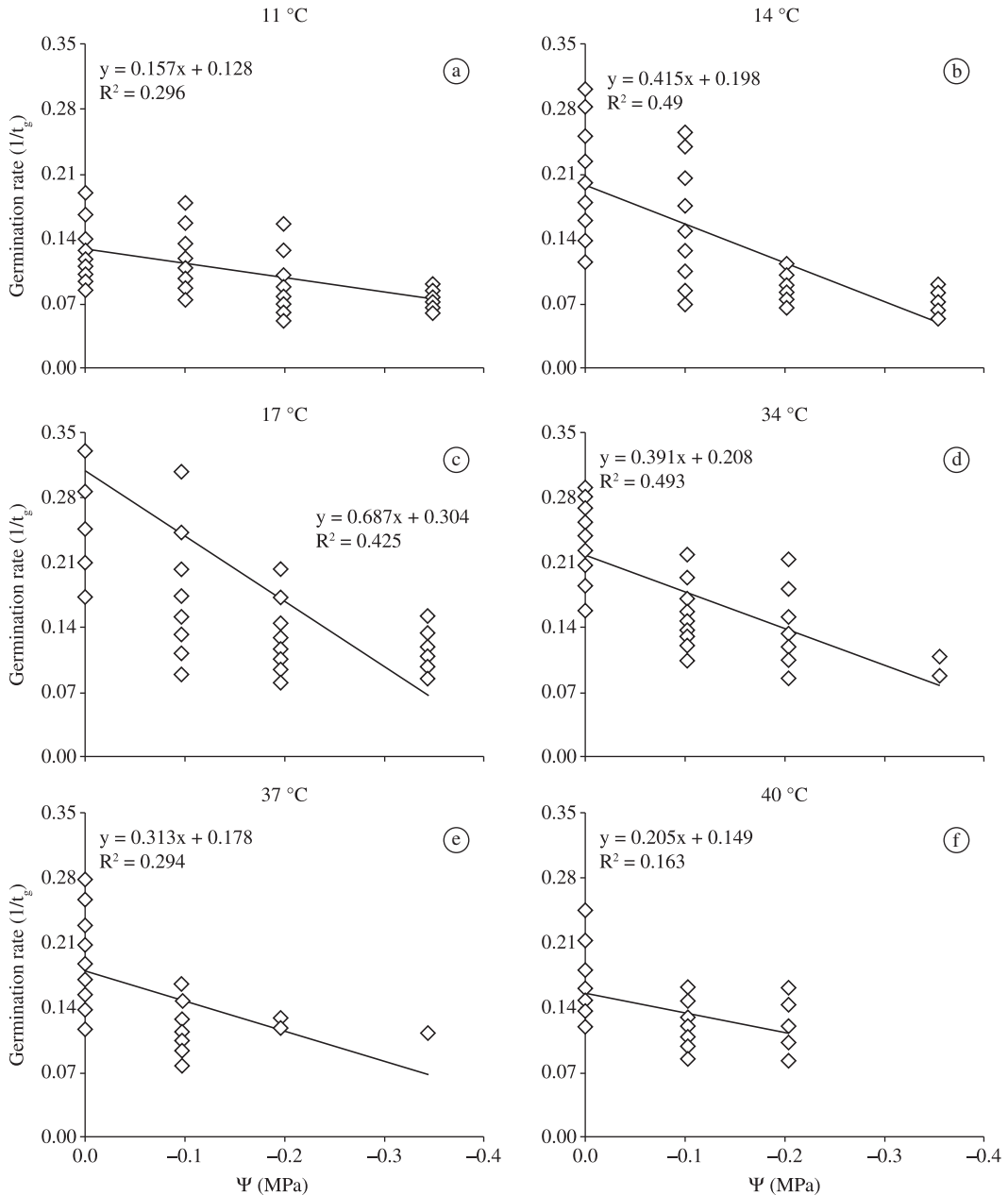
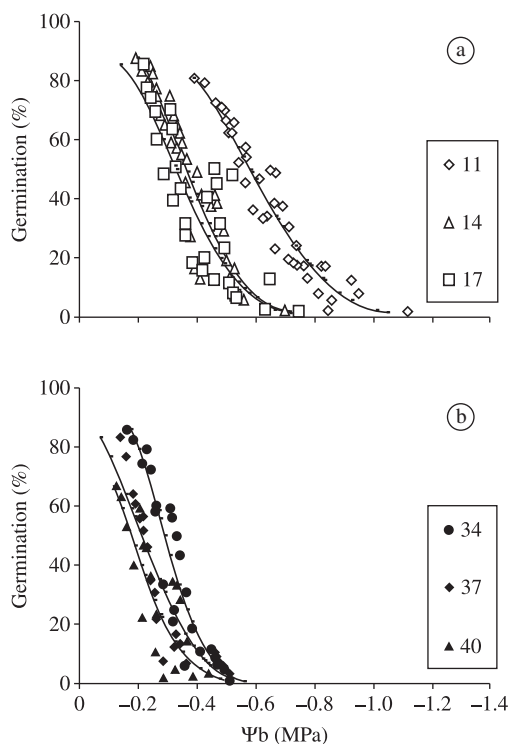


Figure 3. Relationship between seed germination rates, taken as the reciprocal of the time for germination of a given percent fraction g (t_g), and water potential (MPa) in *Hylocereus setaceus* seeds germinated at different temperatures.

Table 2. Parameters of the germination of *Hylocereus setaceus* seeds at supra and infra-optimal temperatures at different water potentials (0, -0.1 MPa, -0.2 MPa and -0.35 MPa).

Model	T (°C)	Parameters			
$\text{Probit}_{(g)} = \{\log[(T - T_b)t_g] - \log\theta_{(50)}\} / \sigma\theta$	11 → 17	$\theta_{(50)}$ (°C day)	T_b (°C)	$\sigma\theta$ (°C)	
		39.3	7.0	18.7	
$\text{Probit}_{(g)} = \{\log[(T + \theta/t_g) - \log T_{c(50)}] / \sigma T_c$	34 → 40	θ (°C day)	$T_{c(50)}$ (°C)	σT_c (°C)	
		77	49.8	5.0	
$\text{Probit}_{(g)} = [\Psi - (\theta_H/t_g) - \Psi_{b(50)}] / \sigma\Psi_b$	11	θ_H (MPa day)	$\Psi_{b(50)}$ (MPa)	$\sigma\Psi_b$ (MPa)	
	14	5.1	-0.58	0.22	
	17	2.1	-0.37	0.15	
	34	1.3	-0.33	0.18	
	37	1.3	-0.29	0.11	
	40	1.3	-0.17	0.14	
$\text{Probit}_{(g)} = \{[\Psi - \theta_{HT} / ((T - T_b)t_g) - \Psi_{b(50)}] / \sigma\Psi_b$	11 → 17	θ_{HT} (MPa °C day)	$\Psi_{b(50)}$ (MPa)	$\sigma\Psi_b$ (MPa)	T_b (°C)
		14.3	-0.38	0.19	7.0
$\text{Probit}_{(g)} = \{[\Psi - k_T(T - T_o)] - (\theta_H/t_g) - \Psi_{b(50)}\} / \sigma\Psi_b$	34 → 40	θ_H (MPa day)	$\Psi_{b(50)}$ (MPa)	$\sigma\Psi_b$ (MPa)	T_o (°C) k_T (MPa / °C)
		1.3	-0.38	0.13	29.5 0.021

**Figure 4.** Relationship between Ψ_b and germination (%) of *Hylocereus setaceus* seeds at infra (a) and supra-optimal (b) temperatures. The solid lines are the respective normal distributions of Ψ_b values among individual seeds.

best fit at each temperature are presented (Table 2). The number of ψ day units decreased from -5.1 MPa day at the temperature of 11 °C to -1.3 MPa day at 17 °C. In the supra-optimal temperature interval a single value of θ_H (= -1.3 MPa day), predicted by the hydrothermal time supra-optimal model across 34-40 °C (Table 2), was used

to fit germination data across different water potentials at each T (34, 37 and 40 °C). It was also observed that the values of $\Psi_{b(50)}$ tend to increase (become less negative) with increasing temperatures, although such variation is not pronounced throughout the thermal interval (Table 2). The distributions of $\Psi_{b(g)}$ values corresponding to the actual daily germination percentages are presented as symbols in Figure 4. The solid lines are the respective normal distributions of Ψ_b values both in the infra-optimum (Figure 4a) and supra-optimum (Figure 4b) interval of T. The curves in the infra-optimum range (Figure 4a) appear to be less inclined than curves in the supra-optimum interval (Figure 4b), as well as the standard deviations of $\Psi_{b(g)}$ at the infra-optimal temperatures tended to be higher as compared to that observed at supra-optimal ones (Table 2). It is also observed that the values of $\Psi_{b(g)}$ decrease (became more negative) the lower the percent fraction of germinated seeds, that is, the “faster” the seed the lower the value of $\Psi_{b(g)}$ (Figure 4).

4. Discussion

In the present work we used *Hylocereus setaceus* seeds stored for about 22 months, and the germinability of the batch was similar to that of newly collected seeds (Simão et al., 2007), suggesting that the seed viability remains unaltered for at least this period of storage. De La Barrera and Nobel (2003) reported that the final germination of *Stenocereus guertaroensis* seeds was maximal for seeds stored between 12 and 28 months and declined after 40 months of storage. The authors observe that the presence of seed dormancy can account for relatively low germinability of recently collected *S. guertaroensis* seeds. The germinability of *H. setaceus* seeds at optimal conditions apparently does not vary over a period of approximately two years from the seed collection. Otherwise, stored *H. setaceus* seeds appear to germinate

in a wider temperature range than newly collected ones (see below), suggesting that the dispersed seeds present relative primary dormancy (Labouriau, 1983).

Hylocereus setaceus seeds are capable of germinating in a relatively wide range of temperatures, with the final germination attaining values between 70 and 100% in the interval of 11 to 40 °C. The germination of *H. setaceus* was rate limited from 11 to 20 °C interval and beyond 30 until 40 °C, since in such thermal intervals the germination rate respectively shifts positively and negatively with temperature. These preliminary results are in accordance with the results obtained by Simão et al. (2007) with newly collected *H. setaceus* seeds. Such authors reported that the germinability of the species is maximal in the range of 15 to 40 °C and the germination rate increases with temperature in the range of 10 to 25 °C and decreases at temperatures above 30 °C. Otherwise, the storage period apparently influenced both the germinability and germination rate response to temperature, since the germinability of newly collected seeds at 10 ± 1 °C was around 5% (Simão et al., 2007) whereas the average germinability at 11 ± 1 °C reported here was 87%, and the germination rate at 20 °C was higher in stored seeds than in newly collected ones. Thus, the temperature of 20 °C was taken as infra-optimal by Simão et al. (2007) and optimal according to the results herein reported. The minimum or base temperature for the germination of *H. setaceus* found in this work (7 °C) is within the range of 5 to 10 °C predicted by Simão et al. (2007), and the median ceiling temperature (corresponding to the fraction 50%) was 49.8 °C, that is within the range of T_c (45 to 50 °C) expected by those authors. From the isothermal assays with *H. setaceus* seeds imbibed in distilled water it is possible to conclude that its germination is optimal in temperatures ranging from 20 to 30 °C, therefore within the optimum range (17 to 34 °C) described for the germination of many other cactacean, with optimal values frequently around 25 °C (Rojas-Arechiga and Vazquez-Yanes, 2000). Dau and Labouriau (1974) reported an optimum temperature of 33 °C for the germination of *Pereskia aculeata*, a cactaceae of fairly common occurrence in the Restinga vegetation, but its germinability drops to zero at 39 °C, whereas *H. setaceus* can germinate at temperatures upper than 40 °C (Simão et al. 2007). The infra-optimum temperatures for the germination of *H. setaceus* are within the range of 7 to 20 °C, and the supra-optimal range is beyond 30 °C.

The germination of *H. setaceus* seeds, as many other species, is sensitive to decreasing values of Ψ in the medium, and both the germinability and the germination rate shift negatively with the reduction of Ψ . In general, the germinability at nominal Ψ of -0.1 MPa and -0.2 MPa did not differ statistically from that in distilled water ($\Psi = 0$), but it was inhibited at -0.35 MPa. These results are similar to that obtained by Ramirez-Padilla and Valverde (2005) with three columnar cactus (*Neobuxbaumia mazcalaensis*, *N. tetetzo* and *N. macrocephala*). The authors reported that a Ψ of

-0.4 MPa inhibited the germinability whereas the germination of seeds incubated in -0.1 MPa PEG solutions did not differ from the distilled water control. Otherwise, the effect of the -0.35 MPa treatment on *H. setaceus* seeds seems to be more pronounced in seeds kept in supra-optimal temperatures, since the lowest values of germinability were observed for seeds incubated at -0.35 MPa at the temperatures of 34, 37 and 40 °C. The germination rate of *H. setaceus* seeds tends to decrease at lower water potentials, and the rate of reduction (the slope of the linear regression of germination rate on Ψ) changed with temperature. The results suggest that the slope is greater toward the optimal temperature range and diminishes toward both minimum and maximal temperatures. Indeed, at the supra-optimal temperature of 40 °C no significant relationship could be found between germination rate of *H. setaceus* seeds and water potentials of 0 to -0.2 MPa. *H. setaceus* appears to be less sensitive to relatively low water potential than *Ficus aurea*, a hemiepiphytic species, which germination is almost nullified in substrate Ψ of -1.5 MPa and lower (Swagel et al., 1997). The seeds of that hemiepiphytic species are deposited by frugivores in humus-filled cavities on potential host trees, and the moisture reaching the humic substrate in leaf bases is retained. According to Swagel et al. (1997) palm leaf base humus potentially holds four times as much water as does open soil, and the moisture content of this humic substrate is 15-16% by mass, translating to a Ψ ranging from -1.0 to -1.1 MPa. Otherwise, the stem water potential in *Quercus agrifolia* ranged from -0.25 to -3.1 MPa, and was negatively correlated with insulation and the percent of the tree canopy exposed to direct overhead sunlight (Swiecki and Bernhardt, 2002). Seeds of *H. setaceus* were not found germinating at the soil surface in the seed collection areas, where the seedlings can be subjected to death during an eventually rapid drying of the substrate. Moreira (unpublished data), working with a dystrophic red latossol with loamy texture, reported that a soil water potential of -0.3 MPa corresponds to a moisture content (% in dry soil) of 25.4%, which is between the permanent wilting point (-1.5 MPa; 22.1% moisture) and field capacity (-0.03 MPa; 31.1% moisture). Thus, regarding this soil, a decrease of the soil moisture as much as 5-6% may be enough to delay and/or inhibits the germination of *H. setaceus* seeds at or near the soil surface. Thus, it is possible that, similar to what occurs in *Ficus aurea*, the germination and seedling establishment only occur on the trunk of the tree host, which provide a more favorable micro-environment with respect to temperature and moisture.

At the supra-optimum temperature range we followed the hypothesis of Alvarado and Bradford (2002) according to which the decrease of both the germination rate and percentage at these temperatures is due to an increase (less negative values) in the $\Psi_{b(g)}$ thresholds for germination, allowing the hydrothermal time model to be extended to the supra-optimal interval. Indeed the values of $\Psi_{b(50)}$ derived from the relationship between

the probits of the accumulated daily germination percentages of *Hylocereus setaceus* and $\Psi - [\theta_H/t_g]$ shift to zero with increasing temperatures, although the median Ψ_b values did not differ significantly to each other within the respective temperature range (if infra or supra-optimum), probably due to relatively high variance of the data. These results differ partially from that obtained by Alvarado and Bradford (2002) with *Solanum tuberosum* seeds, in which the median Ψ_b were relatively constant in the infra-optimal range of T, whereas in the supra-optimum range the Ψ_b values became more positive with temperature. These authors concluded that in the infra-optimal temperature interval, in which $\Psi_{b(50)}$ and σ_{Ψ_b} are relatively unchanged among T, the decrease of t_g is caused by increase of the difference between T and T_b and is reflected in decreasing θ_H values as temperature rises. This feature was observed also in *H. setaceus*, since the hydrotime constant dropped from 5.1 MPa day at 11 °C to 1.3 MPa day at 17 °C. Otherwise, a question remains whether the sensitivity (represented by the variation of $\Psi_{b(g)}$) of *H. setaceus* seeds to Ψ does not change with temperature at the infra-optimum range of T, taking into account the relatively discrepancy among the values of σ_{Ψ_b} at 11 °C and the other two infra-optimal temperatures tested (14 and 17 °C). The standard error (σ) reflects the “spread” of the distributions of the germination times, and the smaller the value of σ , the greater is the effect on germination of any change in dose (Finney, 1971). Contrary to what occurs in the infra-optimum range, at supra-optimal temperatures there is no accumulation of thermal time, and t_g (which is inversely related to the difference between T_c and T) increases with increasing temperatures (Alvarado and Bradford, 2002). If $\Psi_{b(g)}$ becomes less negative with T, then the difference between Ψ and $\Psi_{b(g)}$ decreases, but this decrease is compensated by the increase of t_g due to the effect of the supra optimal T, thus θ_H tends to be constant. A value of θ_H equal to -1.3 MPa day was obtained for *H. setaceus* seeds incubated in different water potentials at the supra-optimum temperature range. Thus *H. setaceus* seeds may require -1.3 MPa day to complete germination at any supra-optimal temperature, and the inverse of θ_H indicates the rate of progress toward radicle protrusion per unit of difference between Ψ and $\Psi_{b(g)}$ (Bradford, 1995). At the supra-optimum range, the values of $\Psi_{b(g)}$ for *H. setaceus* seeds shift positively from -0.29 MPa, at 34 °C, to -0.17 MPa at 40 °C, and such variation reflects in the relatively greater effect of low Ψ (particularly at -0.35 MPa) on germination in that temperature range. In general, the capacity of the model to predict the germination time courses of *H. setaceus* seeds depended on the temperature and water potential. Indeed a relatively poor agreement between the actual and the expected curves was observed in some cases, but the roughly better description of the germination time courses was observed for the water potentials of 0 and -0.1 MPa. The model assumes a common value of T_b for all fractions of the seed population, and that T_b is independent of Ψ and

$\Psi_{b(g)}$ is independent of T, which may not always be the case (Bradford, 1995). Thus, the results presented here suggest that in *H. setaceus* seeds T_b may not be independent of Ψ and that $\Psi_{b(g)}$ may change as a function of temperature at the infra-optimum temperature range. However, further assays are needed in order to test the constancy and independence of T_b and $\Psi_{b(g)}$ in the seed germination of this species.

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