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PHYSIOLOGICAL EFFECTS OF TEMPERATURE AND THERMAL STRESS ON THE SEED GERMINATION OF *STRYPHNODENDRON POLYPHYLLUM*

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TAMBELINI, M. & PEREZ, S. C. J. G. A. 1999. Physiological effects of temperature and thermal stress on the seed germination of *Stryphnodendron polyphyllum*. The thermal effects brought about by temperature on the seed germination of *Stryphnodendron polyphyllum* were studied in relation to the germination percentage and relative frequency distribution of germination along the incubation time. *Stryphnodendron polyphyllum* seeds were found to be eurithermic, having the following cardinal temperatures: the minimum between 5 and 10 °C, the maximum between 40 and 45 °C, the optimum between 25 and 30 °C. The germination rate was temperature dependent. However, the distribution of relative frequency of germination did not fit the adjusted gaussian and did not give good linearity in the Arrhenius graphs of their isothermal germination rates. Hence, in these seeds the thermal communication between the environment and the seed growth effector is made through a temperature signal superimposed upon the random thermal noise at all germination isotherms. The absolute values of enthalpy activation of germination ($\Delta H^{\#}_{n}$) were less than 12 Kcal mol^{-1} between 20 and 30 °C, 12 and 30 Kcal mol^{-1} at 15 °C and higher than 30 Kcal mol^{-1} above 35 °C. Therefore, the germination of the seeds was only limited by diffusion process between 2 and 30 °C. Below 15 and above 35 °C phase transitions and protein denaturation occurred. The lowest values of informational entropy occurred between 25 and 30 °C, showing a higher synchronisation of the germination process. Seeds without imbibition were resistant to exposure at 70 °C for 24 h, without showing a significant reduction in the rate and percentage of germination. Thermal stress of imbibed seeds at 50 °C for 24 h gave only 10% germination.

Key words: Germination - temperature - thermal stress - Brazilian "cerrado".

TAMBELINI, M. & PEREZ, S. C. J. G. A. 1999. Kesan fisiologi suhu dan tegasan terma terhadap percambahan biji benih *Stryphnodendron polyphyllum*. Kesan terma yang dibawa oleh suhu terhadap percambahan biji benih *Stryphnodendron polyphyllum* dan kaitannya dengan peratus percambahan dan kekerapan relatif taburan percambahan di sepanjang tempoh pengeraman dikaji. Biji benih *Stryphnodendron polyphyllum* didapati lebih euritemik, mempunyai suhu utama yang berikut: minimum antara 5 dan 10 °C, maksimum antara 40 dan 45 °C, optimum antara 25 dan 30 °C. Kadar percambahan bergantung kepada suhu. Bagaimanapun, taburan kekerapan relatif percambahan tidak sesuai dengan taburan biasa yang diselaraskan. Ia juga tidak memberikan kelinearan dalam graf Arrhenius bagi kadar percambahan isotermanya. Oleh yang demikian, dalam biji benih ini, komunikasi terma antara alam sekitar dan penindak pertumbuhan biji benih dilakukan melalui isyarat suhu yang ditindih di atas bunyi bising terma secara rawak pada kesemua percambahan isotem. Nilai mutlak pengaktifan entalpi percambahan ($\Delta H^{\#}_{n}$) ialah kurang daripada 12 Kcal mol^{-1} antara 20 dan 30°C, 12 dan 30 Kcal mol^{-1} pada 15 °C dan lebih tinggi daripada 30 Kcal mol^{-1} di atas 35 °C. Oleh itu, percambahan biji benih hanya dihadkan oleh proses resapan antara 2 dan 30 °C. Di bawah 15 dan di atas 35 °C wujud peralihan fasa dan penyahaslian protein. Nilai terendah entropi bermaklumat terjadi antara 25 dan 30 °C, mewujudkan penyelarasan yang lebih tinggi dalam proses percambahan. Biji benih tanpa pendapan tahan kepada pendedahan pada 70 °C selama 24 jam, tanpa menunjukkan pengurangan bererti dalam kadar dan peratus percambahan. Tegasan terma biji benih yang dipendap pada suhu 50 °C selama 24 jam hanya memberikan 10% percambahan.

Introduction

Stryphnodendron polyphyllum Mart, a member of the Leguminosae, occurs in Brazilian "cerrado" and is locally known as "barbatimão". Its trunk has a high content of tannin that can be used in tanning and the bark has medicinal properties. The tree is also ornamental, and may be used in landscape gardening and in urban forestry; its wood is good for construction. This species is also recommended for mixed plantation in degraded and permanent conservation areas.

According to Labouriau (1983), it is important to determine the extreme cardinal temperatures (T_m and T_M) and the optimum range of germination percentage to understand the biology and ecology of the species.

The main physiological interest in studying the temperature dependence of germination is the search for limiting factors and partial processes of seed germination.

Temperature effects can be determined from changes in the percentage, rate and relative frequency of germination (Labouriau & Agudo 1987). The extreme temperature limits of seed germination are easily determined empirically and provide biogeographical and ecological information (Labouriau 1983).

Since the overall process is strongly affected by temperature, its mechanisms may be subjected to thermobiological analysis, and germinating seeds provide the basis for many thermobiological investigations (Labouriau 1983).

One approach to studying the temperature dependence of the germination rate is to determine the activation enthalpy. This makes possible the identification of the temperature intervals in which the germination is limited by photochemical reactions (very small values), by diffusion processes ($/\Delta H^{\#}/<12$ Kcal mol^{-1}), by phase transitions or thermal transconformation of proteins ($/\Delta H^{\#}/>30$ Kcal mole^{-1}). Another approach is to evaluate the synchronisation of individual seed germination, using the synchronisation index, that expresses the informational entropy or uncertainty associated with relative frequences distribution at germination (Labouriau & Agudo 1987).

According to Heydecker (1973), an environmental factor present at extreme values may be of considerable biological stress. However, thermal stress could promote the germination of seeds with hard coats (Egley 1989).

Thus, due to the lack of information about tropical wood species, the objective of this study was to determine the cardinal germination temperatures and the impact of thermal shock on the germination of *Stryphnodendron polyphyllum* seeds.

Material and methods

Seeds of *Stryphnodendron polyphyllum* were collected from "cerrado" vegetation areas. The seeds were then scarified with 98% concentrated sulphuric acid for 60 min (Tambelini 1994), washed in water, disinfected in 2% sodium hypochlorite and finally washed in distilled water. Four replications of 50 seeds were used for each treatment and incubated in Petri dishes (15 cm diameter) using filter paper moistened with 15 ml of fungicide solution (Benomyl). Observations were daily recorded and germinated seeds (with radicle ≥ 2 mm length) were removed as soon as detected. The cardinal germination temperatures were determined under growth chamber conditions with constant temperatures, ranging from 5 to 45 °C, with intervals of 5 °C.

The thermal stress was evaluated at 3 levels: a) intact seeds were exposed at 50, 60, 70 and 80 °C for 48 h; b) scarified and unimbibed seeds exposed at 50 °C for 24 h and 50, 60, 70 and 80 °C for 48h; c) scarified and imbibed seeds were exposed at 50 and 60 °C for 24 h. The seeds were incubated at 30 °C after the thermal shock exposure.

The parameters of germination were estimated using expressions described by Labouriau and Agudo (1987):

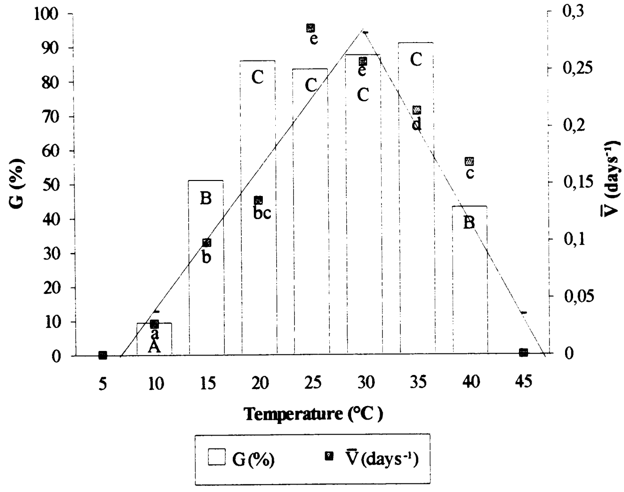
- germination rate, $v = 1/t$ (in day^{1})
- relative frequency distribution, $f_i = n_i/\Sigma n_i$ (where n_i = number of seeds germinated between observations t_{i-1} and t_i);
- enthalpy, $\Delta H^{\#}_{n} = [R\,(T_m + T_M).(\theta - T).T]/[T - T_m).(T_M - T)]$ (where R is the universal gas constant, T is the incubation temperature in the Kelvin scale and θ is the average between T_m and T_M);
- informational entropy of activation, $E = -\Sigma f_i . 1g_2 f_i$ (where $f_i = n_i/N$).

The germination percentages were arcsin transformed and the results were compared using *F*-test (Sokal & Rohlf 1980).

Results and discussion

Temperature effects

Germination of *Stryphnodendron polyphyllum* seeds occurs from a minimum between 5 and 10 °C up to a maximum between 40 and 45 °C with optimal range from 25 to 30 °C (Figure 1). At 5 °C the seeds showed no germination even after two months at this temperature and at 45 °C the seeds presented a black coat with an extrusion of gelatinous substance. The highest rates of germination were observed at 25 and 30 °C without significant differences among them. Above and below this optimum range, the rate decreased significantly.

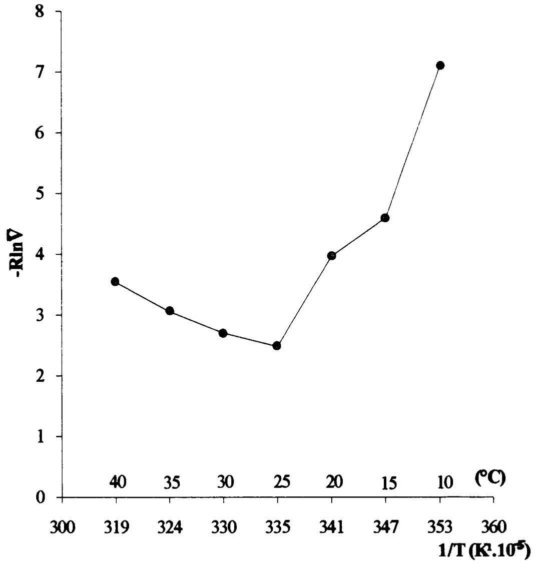


(Continuous line - V theoretical points: $V = 0.01358\ T - 0.09410$ $r = 0.9551$; $V = -0.01628\ T + 0.7700$, $r = -0.9362$). Means followed by the same letters do not differ at $p<0.05$.

Figure 1. Influence of different temperatures on germination percentage (G) and germination rate (V) in *Stryphnodendron polyphyllum* seeds

Due to the germination temperature range, *Stryphnodendron polyphyllum* may be classified as a tropical species, having a high temperature tolerance, with a maximal limit of or above 35 °C, and a minimum limit generally above 5 °C (Okusanya 1978 in Perez 1988). Similar cardinal temperatures have been found for other "cerrado" species like *Magonia pubescens* (ranging from 10 to 40 °C) (Joly *et al.* 1980) and *Dimorphandra mollis* (ranging from 12 to 33°C) (Zpevăk 1994).

An approach to identifying the activation energy of this process is in the use of the Arrhenius equation. This type of analysis assumes that a physiological process does not occur unless the system overpasses an energy threshold and that the distribution of energy within the system takes place only through elastic molecular collisions. In this case, the graphs should give only straight lines. There are physiological processes in which this condition is indeed fulfilled, but in many instances, the Arrhenius plots are definitely curved, as the germination rates of *Stryphnodendron polyphyllum* seeds show (Figure 2). Several other species that present a non-linear Arrhenius plot are *Salvia hispanica* (Labouriau & Agudo 1987), *Prosopis juliflora* (Perez & Moraes 1990) and *Dimorphandra mollis* (Zpevăk 1994).



(V̄: rate of germination; R: universal gas constant (0.08205 atm.K^{-1} mol^{-1});
T: absolute temperature (K)

Figure 2. Arrhenius plot of germination rate in *Stryphnodendron polyphyllum* seeds

It is apparent that seed germination rates of this species must be analysed by another approach, like the partition of energy within the seed system (Labouriau 1983). The Eyring theory reported by Perez and Moraes (1990) postulated that a) the system must have several degrees of freedom, among which its energy is partitioned, b) the system must be always in equilibrium with its environment, and c) the process cannot take place unless the system has overpassed a certain potential energy barrier. The same theory says that there are two parcels of energy spent in the activation process: enthalpy and entropy. In seed germination systems with linearly temperature dependent rates, high variations of enthalpy activation take place near the temperature limits, and at the optimum range the enthalpy variations present low values.

The absolute values of enthalpy variation of *Stryphnodendron polyphyllum* seed germination ($\Delta H^{\#}_{n}$) (Table 1) were lower than 12 Kcal mol^{-1} between 20 °C (293K) and 30 °C (303K). Values between 12 and 30 Kcal mol^{-1} take place at 15 °C (288K), and above 35 °C (308K) the values were higher than 30 Kcal mol^{-1}. Thus, the germination of *Stryphnodendron polyphyllum* seeds would only be limited by the diffusion process between 20 and 30 °C. Below 15 °C and above 35 °C phase transitions and protein denaturation may occur.

Table 1. Temperature dependence on net enthalpy variation ΔH^{*}_{n} and entropy (E) variation for germination activation in *Stryphnodendron polyphyllum* seeds

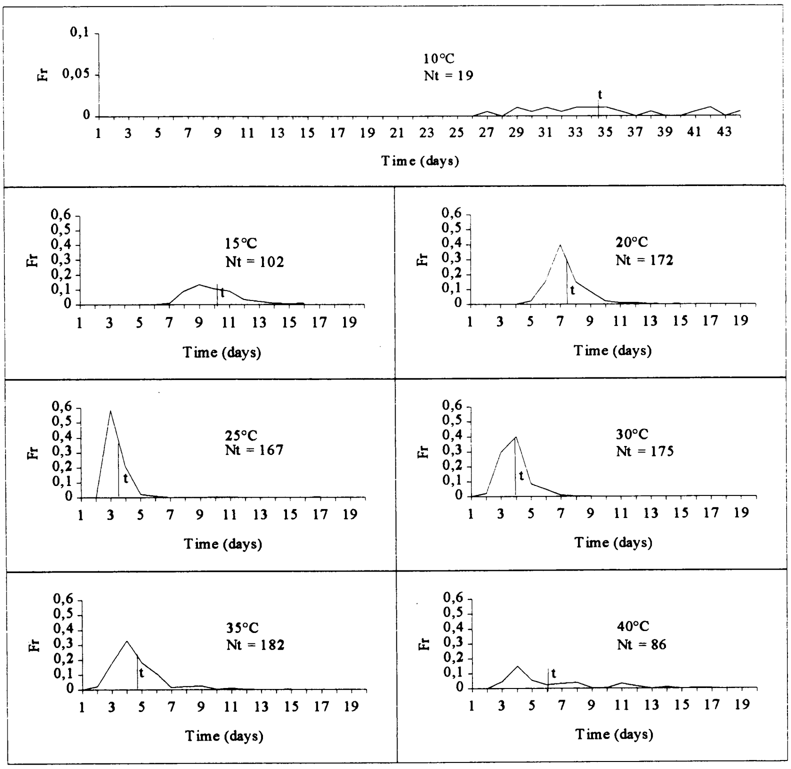
T (°C)	T(K)	V	T (°C)	1/T ($K^{-1}.10^{-5}$)	-Rln V	ΔH^{*}_{n} ($Kcal/mol^{-1}$)	E (bits)
5	278	No germination					
10	283	0.028	5–10 °C	353	7.10		2.17 A
15	288	0.099	10–15 °C	347	4.59	21.018	2.37 A
20	293	0.135	15–20 °C	341	3.97	7.363	2.00 A
25	298	0.286	20–25 °C	335	2.48	- 1.185	1.10 B
30	303	0.256	25–30 °C	330	2.70	- 10.327	1.75 AB
35	308	0.214	30–35 °C	324	3.06	- 31.385	2.36 A
40	313	0.168	35–340 °C	319	3.54		2.57A
45	318	No germination					

Means followed by the same letters do not differ at $p<0.05$.

The approach using the theory of absolute reaction rates requires the understanding of the physiological meaning of large values of activation enthalpy. Two phenomena show activation enthalpy of this magnitude: phase transitions and protein denaturation. Either one may be the cause. Phase transitions, i.e. the melting of lipids, has previously been suggested to be involved in several physiological phenomena with very high activation energies. The denaturation of an amylase and an oxidase has been suggested as the cause of the decrease in germinability (with very high Q10 values) and of the lowering of the activity of these enzymes when seeds are submitted to thermal shocks (Labouriau 1978).

Phase transitions, thermodenaturations and damage to cytomembranes have been all presented by Cohen (cited by Labouriau 1978) as possible causes of high values of activation enthalpy in the germination of lettuce seeds. An investigation of the leakage of amino acids from incubated seeds of several species showed that the leakage is enhanced at supra-optimal incubation temperatures, but the results are consistent both with the melting of lipids and with the denaturation of proteins of cytomembrances. One way of discriminating between these two possibilities is in the study of the effects of deuterium oxide on isothermal seed germination, which has a protective action against protein thermodenaturation (Labouriau 1978). This we did not do.

Whenever seed germination rates are temperature dependent, the frequency distributions along the time of isothermal incubation must be different for different temperatures. Thus, for *Stryphnodendron polyphyllum* the graph (Figure 3) shows the temperature specification of the embryo growth which is necessary and sufficient for its emergence from the seed coat. In the isothermals from 15 to 30 °C, they are unimodal. However, polymodality is clearly shown at 10, 35 and 40 °C indicating a physiological heterogeneity of seeds as to their individual germination. Furthermore, the relative position of the average germination time and the main mode can show whether the heterogeneity is due to a minority of slower germinating seeds (optimum range) or to a minority of faster germination seeds (extreme cardinal temperatures). In this study, the medium time is displaced to the right of the main mode, within the optimum range and at the temperatures near to maximum (Figure 3). This may be due to a few seeds presenting slower germination (optimum range), or due to many seeds with slower germination (near to maximum).



The Fr values represent the replications sum and 't' represent the mean germination time (Nt = total number of germinated seeds)

Figure 3. Relative frequency distribution (Fr) on seed germination of *Stryphnodendron polyphyllum* along the time of isothermal incubation for different temperatures

Since relative frequency distribution describes the specification of seed germination by external temperatures, it can be looked upon as expressing a thermal communication between the environment and seed growth effector. The measurement of the information content of this communication is given by the uncertainty value attached to the distribution (Shannon 1948, in Labouriau 1978). Thus, two situations can be envisaged. Either this thermal communication follows the mechanism of random molecular shocks, or else there is a more organised system of heat transmission superimposed upon the random molecular collisions.

The quantitative discrimination between the two alternatives is made possible by a theorem (Shannon 1948, in Labouriau 1983) which states that for a given variance, the gaussian is a distribution of maximum uncertainty. In the case of *Stryphnodendron polyphyllum* seeds, the graphs (Figure 3) did not fit adjusted normal curves and so, the thermal communication would be made through a temperature signal superimposed upon random thermal noise.

Seeds of *Stryphnodendron polyphyllum* presented the lowest value of informational entropy at 25 and 30 °C, and the highest values in 15 and 40 °C (Table 1). At 25 and 30 °C, the system has higher information conditions, presenting high rates and germination percentages in these isotherms.

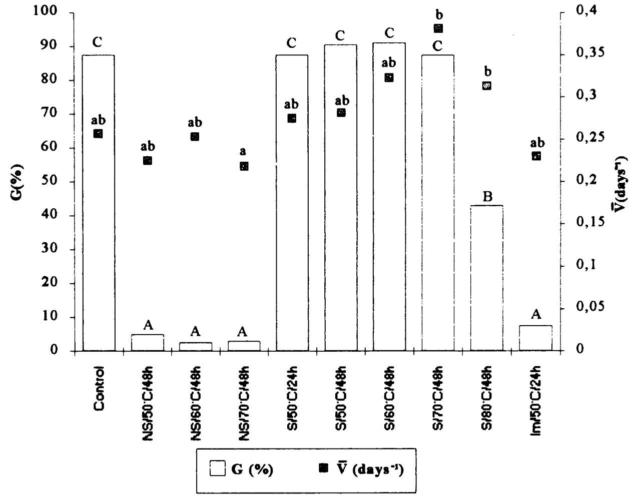
Thermal stress

According to Rolston (1978), Egley (1989) and Bell *et al.* (1993), thermal stress may promote the germination of hard coat seeds and increase the germination percentage. Perez and Fanti (1996) achieved a higher rate and percentage of germination using thermal stress on *Peltophorium dubium* seeds. However, this procedure is not effective in breaking the mechanical dormancy of *Stryphnodendron polyphyllum* seeds without scarification pretreatment, when exposed to a thermal stress at 50, 60, 70 °C (Figure 4). Scarified seeds were resistant to thermal stress up to 70 °C for 48 h, but a significant reduction of the radicle length and coat alterations were observed. Exposure of the scarifieds seeds to 80 °C for 48 h promoted a leakage of gelatinous substances throughout the coat and a reduction in the viability and radicle length. Imbibed seeds were sensitive to thermal stress, presenting germination percentage as low as 10%. Sometimes, the thermal stress could affect the seedling development adversely (Mayer & Poljakoff-Mayber 1989) as has been obtained in *Prosopsis juliflora* (Perez & Moraes 1990) and *Leucaena leucocephala* (Cavalcante & Perez 1995).

A probable effect of high temperatures on germination is an increased respiration rate, leading to a high energy production, available for the growth (Okusanya 1980). This would be the reason for the slight increase in the rates observed in scarifieds seeds exposed to 60 and 70 °C.

Conclusion

The germination of *Stryphnodendron polyphyllum* seeds is a eurithermic process, occurring from a minimum between 5 and 10 °C up to a maximum between 40 and 45 °C, with optimal range from 25 to 30 °C. It is limited by diffusion process between 20 and 30 °C, and probably by phase transitions or proteins denaturation below 15 and above 35° C. Thermal stress of 50–70 °C is not effective in breaking the mechanical dormancy of *Stryphnodendron polyphyllum* seeds. *Stryphnodendron polyphyllum* scarified seeds are thermal resistant until 70 °C during 48 h when not imbibed, but imbibed seeds already show thermal stress when exposed to 50 °C for 24 h.



(NS: seeds without scarification; S: seeds after scarification; Im: imbibed seeds).
Means followed the same letters did not differ at $p<0.05$.

Figure 4. Influence of distinct thermal stresses on germination percentage (G) and germination rate (V) in *Stryphnodendron polyphyllum* seeds. The values of G and V represent the replications means

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