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Seed Germination in *Miconia theaezans* (Bonpl.) Cogniaux (Melastomataceae)

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ABSTRACT

The effects of light and temperature were studied on the seeds of Miconia theazeans *by isothermic and alternating temperature incubations. The optimum temperature for seed germination was determined by final percentage and germination rates as located in the range of 27.5 to 30 °C and by germination kinetics at the range of 19.5 to 30 °C. The germination was dependent on diffusion processes. The minimum and maximum temperatures were 12.5-15°C and 32.5-35°C, respectively. The seeds showed strong light dependence for germination with the necessity of daily 4-6 h white light irradiation for the maximum induction of germination. However, under 30-20 °C alternating temperatures, daily 2 hours white light was enough to induce germination and attained maximum under 4 h photoperiod. The results indicated that* M. theazeans *presented characteristics of early successional species.*

Key words: Light, seed germination, temperature

INTRODUCTION

Decrease in the biodiversity of plants in tropical forests is the main consequence of human activities, especially in the Atlantic forest. The knowledge of the dynamics of reproduction responses, such as pollination, dispersion and germination of seeds in disturbed areas is important for the management and conservation of natural forests and for reforestation (Bruna 1999). Regeneration processes in a tropical forest are associated to the dynamic of secondary succession with colonization by species with ecophysiological adaptation to different environmental conditions (Gómez–Pompa et al. 1991).

Gaps in the canopy formed by the fall of one or more trees are important for the maintenance of the biodiversity of species in a tropical forest (Hartshorn, 1980). In this case, the maintenance of biodiversity is related to the number of species that occur in the forest and the presence of seeds in the soil seed bank. Pioneer species present characteristics which are important for the colonization of gaps of the forest (Whitmore 1990). Whitmore (1989) classified plant species in two successional categories: i. pioneer and ii. non-pioneer species. Earlier, Budowski (1965) classified plant species in four categories: i. pioneers, which are found in large gaps of the canopy, with fast growth, producing high number of small seeds which are dispersed by animals forming soil seed bank; ii. early secondary species, similar to pioneer species, but found in smaller gaps; iii. late secondary and iv. climax species

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found in the shade of the canopy with slow development rate.

Species in the secondary succession can be classified in three distinct categories: i. specialists of large gaps, with dormant seeds which germinate only under high temperature or high light fluences and present shade avoidance responses; ii. specialists of small gaps, with seeds that germinate under the canopy and iii. specialists of canopy, with seeds and seedlings which do not need full sun for germination and seedling development (Denslow 1980). Seeds of pioneer species, found in the soil seed bank, require light with high fluence and R:FR ratio, high temperature or alternating temperature for germination and those conditions can be found in gaps of forest canopies (Kyereh et al. 1999, Kageyama et al. 1990, Bazzaz 1984).

Miconia theaezans (Melastomataceae) is found from Central America to State of Santa Catarina in Brazil; in São Paulo State it is found in gallery forests. *M. theaezans* is classified as early secondary species (Knobel 1995). It produces fruits which are eaten especially by bird, responsible for dispersion of its seeds. Baider et al. (1999) working with composition of soil seed bank at Atlantic forest and colonization of natural gaps, found species mainly from the Melastomataceae, especially *Miconia, Leandra* and *Rapanea*. Melastomataceae species are reported to be important for the forest restoration programs (Macedo 1993) to improve biodiversity of the resulting forest by attracting different seed dispersers.

The aim of the present work was to study the cardinal and the optimal temperatures as characteristics of *M. theaezans* seed germination. The interaction of temperature and light was also analyzed for determination of seed behavior to give information for the management and conservation of *M. theaezans* in natural forests and for reforestation of disturbed areas projects.

MATERIAL AND METHODS

Seeds of *M. theaezans* (Bonpl.) Cogniaux were obtained from fruits harvested in an area of Atlantic Forest at São Bernardo do Campo city, State of São Paulo, Brazil. The fruits were ground in a becker with the aid of a pistil and water. The seeds with fruit parts were cleaned with a sieve and dried at a constant temperature of 25 °C. Dried seeds were stored inside a stoppered jar and maintained at 5 °C during one year. The experiments started just after the seeds were collected. For germination tests, 50 mm diameter Petri dishes were used with two layers of water imbibed filter paper inside. The Petri dishes were kept inside dark plastic boxes for dark treatments and colorless plastic boxes for light treatments. Constant temperatures were obtained with incubators. Due to the small seed size, around 200 seeds were put inside each Petri dish, as proposed by Cone and Kendrick (1985) for seeds of *Arabidopsis thaliana* (L.) Heynh.

Germinated seeds were counted daily and seeds with at least 1mm long roots were considered as germinated. Dark incubated seeds were observed under a dim green safe light (Amaral-Baroli and Takaki 2001). The experiments were finished when seeds did not germinate at least for 5 successive counts and last 50 days under sub and supra optimum temperatures.

Different temperatures were tested (10-35 °C with 2.5 °C intervals) and the photoperiod effect was tested at 27.5 °C. Alternating temperatures (30-20 °C, 25-20 °C; 22.5-20 °C; 35-20 °C e 30-15 °C) were tested with 12 h photoperiod. At 20-30 °C alternating temperatures different photoperiods were tested. White light at 8.93 $\mu mol.m^{-2}.s^{-1}.nm^{-1}$ was obtained with the aid of two 15 W day light fluorescent lamps and measured with a LI-1800 spectroradiometer (LI-COR, U.S.A.).

The final percentage (G), rate of germination (GR) and germination synchronization index (SI) were calculated according to Labouriau and Agudo (1987). Analysis of kinetics of seed germination was conducted according to Labouriau and Osborn (1984) using the variation in the enthalpy of activation of seed germination $\Delta H\#\eta=\{[R.T(\theta-T).(Tm-TM)]/[(T-Tm).(TM-T)]\}$; where R is the gas universal constant of 1.987 cal; Tm the minimum temperature, TM the maximum temperature of germination, θ the harmonic mean between Tm and TM and T the temperature (°K). The effects of light and temperature were determined by the analysis of variance followed by a Student-Newman Keuls or by Kruskal-Wallis test (Zar, 1998), and an alpha level of 0.05 was adopted as significant in all circumstances. Before the analysis, the germination data were transformed to $\arcsin\sqrt{(\%:100)}$. The regression lines for effect of photoperiod on germination of seeds incubated at 27.5 ºC and 30-20 ºC alternating temperature were obtained using the logistic function, according to Hsu et al. (1984).

RESULTS AND DISCUSSION

Seeds of *M. theaezans* exposed to constant temperatures below 15 °C and above 32.5 °C did not germinate. The high values of G were observed between 15 °C and 32.5 °C, with no significant differences observed within this range (Table 1).

The optimum temperature for germination of tropical species usually are higher than those observed for temperate species, and the seeds of several tropical species can germinate in a broad range of temperatures (Kozlowski, 2002).

Table 1 - Seed germination of *Miconia theazeans* under different constant temperature and continuous white light. Means ± standard deviation for: seed germination G (%); (GR) mean germination rate and synchronization index (SI). Means followed by the same letter are not significant at 5% level.

Temperature (°C)	G (%)	(GR)	(SI)
10.0	0	-	-
12.5	0	-	-
15.0	89.0 ± 9.40[a]	0.036 ± 0.0002[e]	2.55 ± 0.13[a]
17.5	90.0 ± 1.87[a]	0.062 ± 0.0022[d]	3.41 ± 0.02[a]
20.0	96.3 ± 4.57[a]	0.077 ± 0.0029[c]	2.17 ± 0.20[a]
22.5	97.4 ± 1.29[a]	0.111 ± 0.0007[b]	2.11 ± 0.16[a]
25.0	95.3 ± 2.68[a]	0.111 ± 0.0012[b]	1.94 ± 0.23[a]
27.5	97.1 ± 1.95[a]	0.115 ± 0.0018[a]	2.36 ± 0.06[a]
30.0	98.2 ± 1.30[a]	0.114 ± 0.0009[a]	2.05 ± 0.17[a]
32.5	97.4 ± 0.88[a]	0.105 ± 0.1052[b]	2.24 ± 0.08[a]
35.0	0	-	-

An increase in the mean germination rate was observed as temperature increased from 15 to 22.5 °C. No difference was observed in mean germination rate between 27.5 and 30 °C, as well as between 22.5 and 25 °C. However, these values were significantly different from any other tested isotherms. According to Labouriau and Osborn (1984), the rate of seed germination was limited only by diffusion processes at $|\Delta H\#n| < 12$ $kcal.mol^{-1}$ which in seeds of *M. theazeans* occurred under temperatures ranges of 19.5 to 30.0 ºC (Fig. 1). Thus, the optimum temperature for the germination of seeds of *M. theazeans* must be in this range of temperature. The statistical analysis indicated that the optimum temperature was between 27.5 and 30.0 °C, which could be considered high confirming the behavior of the species which colonized open areas. Similar results were obtained by Ferraz-Grande and Takaki (2001) in the seeds of *Dalbegia nigra* Allem. with optimum temperature at 30.5 °C and range of temperature with seed germination from 15 to 40 °C, and by Andrade (1995) in the seeds of *Tibouchina benthaminana*, *Tibouchina grandifolia*, *Tibouchina moricandiana* and *Leandra breviflora*, with 30°C as optimum temperature.

Souza and Válio (2001) reported that seeds of pioneer species of *Solanum granuloso-leprosum* Dun., *Trema micrantha* (L.) Blume and *Cecropia pachystachya* Trec., when exposed to continuous dark at 25 °C did not germinate, but under the same temperature and continuous white light, the germination of *C. pachystachya* was 84%, while *S. granuloso-leprosum* showed 4.8% of germination and *T. micrantha* 2.5%.

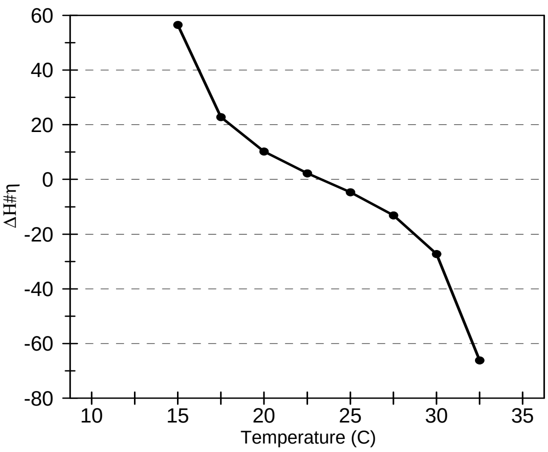

Figure 1 - Variation in enthalpy of activation (ΔH#η) of seed germination in *M. theazeans*

Present data showed that at constant 27.5 °C, a two hours daily light treatment were not enough to promote germination, and under a photoperiod of 4 h only 14.1% of the seeds germinated. Above this photoperiod, an increase in the percentage of germination was observed with increase in the photoperiod. The highest percentage of germination was found between the photoperiods of 6 and 12 h and in the later condition 97.5% of the seeds germinated (Fig. 2). The values of mean germination rate increased with increase in the photoperiod and small decrease was observed at 12 h photoperiod. The highest mean germination rate was observed at 10 h photoperiod, and all the values differed significantly from each other (Table 2).

Alseis blackiana Hemsl. is an abundant species in the canopy forest, with seedlings that can only be found in gaps. Its germination was tested in a gap and under the canopy; the results showed a higher germination in the gap (40 ± 9%) than under the canopy (8 ± 2%).

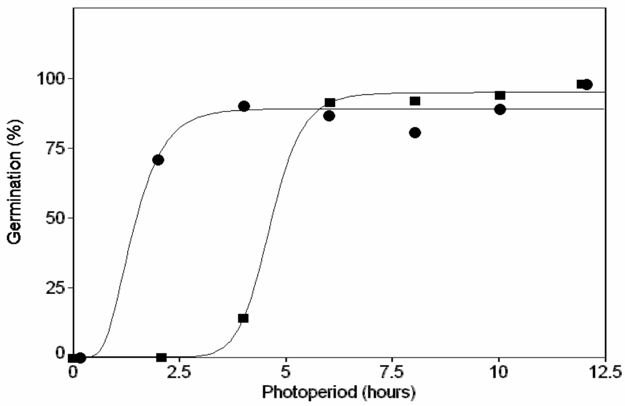

Figure 2 - Effect of photoperiod on germination of seeds of *Miconia theaezans* incubated at 27.5 °C (-■-) and under alternating 30-20 °C (-•-). Lines represent the regression obtained by logistic function with r^2=0.998 F=873.5 for (-■-) and r^2 =0.978 F=91.7 for (-•-).

Thus, this species seemed to be able to survive under the canopy leading to the high capacity of light absorption (even from occasional sun flecks) and the fast growing in the initial period (Dalling et al. 2001).

The lowest values for the germination synchronization index of *M. theazeans* were found under a photoperiod of 10 and 12 h. No difference was observed between the values obtained at 4 and 6 h and between 6 and 8 h. Germination under a photoperiod of 4 hours was slow and less synchronized than under 12 h photoperiod, when the seeds germinated in a higher and synchronized germination.

Ekstam and Forsebay (1999) reported that seeds of *Phragmites australis* (Cav.) Trin. Ex Steudel and *Typha latifolia* L. germinated after dispersion under low mean daily temperatures, as long as the range of variation was wider. However, the seeds that were dispersed in unfavorable seasons, such as in autumn and winter, when mean daily temperatures were lower and with narrow range, could not germinate until the soil was warmed and the range of thermal variation increases. Benvenuti et al. (2001) observed that alternating temperatures had an ecological relevance for seedling emergence under natural conditions, and that daily fluctuation in temperatures could be more important as an environmental clue than light for triggering the germination of seeds that are close to the soil surface.

Bazzaz and Pickett (1980) reported that the temperature in the forest soil showed small difference from air temperature. This difference can be substantial in gaps, where daily variation in temperature range from 25 °C at night up to 42 °C during the day. Bazzaz (1984) mentioned that seeds of the initial stage of secondary succession germinated under micro clime conditions associated with alternating temperatures. These ideas were corroborated by Kyereh et al. (1999) who indicated that the gap formation is associated with changes in the range of temperature and in the maximum temperature, as well as in the water availability in the soil. Evidently, light sensitivity of the seeds was a feature of several pioneer species and was associated with the small seeds that usually composed the soil seed bank. *Psidium guajava*, a gap colonizing species, present light sensitive seeds which germinate in darkness under alternating temperatures, while under constant temperature they germinate only under white light (Sugahara and Takaki 2004). Thus gap and open area colonizing species can present different behavior under different temperatures.

Table 2 - Seed germination of *Miconia theazeans* under constant 27.5 °C and different light regimes. Means ± standard deviation for: seed germination G (%); (GR) mean germination rate and synchronization index (SI). Means followed by the same letter are not significant at 5% level.

Light regimes (h)	(GR)	(SI)
0	-	-
2	-	-
4	0.034 ± 0.0009^{d}	3.27 ± 0.47^{a}
6	0.062 ± 0.0013^{c}	2.95 ± 0.23^{ab}
8	0.073 ± 0.0018^{b}	2.67 ± 0.17^{b}
10	0.118 ± 0.0033^{a}	2.04 ± 0.18^{c}
12	0.098 ± 0.0007^{b}	1.88 ± 0.22^{c}

Alternation of 35-20 °C did not promote germination of *M. theazeans* seeds than isothermic incubations. Germination was higher at an alternating 30-20 °C than other treatments. No difference was found between 22.5-20 °C and 25-20 °C and between 22.5-20 °C and 30-15 °C alternating temperatures (Table 3). The seeds did not germinate in the dark under all treatments. Mean rate of germination of *M. theazeans* differed statistically among all alternating temperature treatments and higher values were observed under an alternation of 22.5-20 °C and 25-20 °C, while the lowest value was observed under the alternation of 30-20 °C.

Under 30-20 °C alternating temperatures, high values of percentage of germination were observed between photoperiods of 4 to 12 h (Fig. 2). However, two hours photoperiod was enough to induce high percentage germination while at 27.5 °C isothermic incubations two hours photoperiod did not induce germination. Data for mean rate of germination showed that the highest value was observed under a photoperiod of 12 h, and that this value differed from all other values. On the other hand, no significant differences were observed between the photoperiods of 6 and 10 h and between the photoperiods of 2 and 8 h (Table 4). In some species such as *Trema micrantha*, germination was promoted under alternating temperatures and the mean germination rate was higher at alternating temperatures of 20-30 °C and e 30-40 °C (Castellani and Aguiar, 2001). Our results indicate that seeds of *M. theaezans* present increase in light sensitivity when under alternating temperatures, a condition present in gaps and open areas (Bazzaz and Pickett 1980).

Table 3 - Seed germination of *Miconia theazeans* under alternating temperatures. Over 12h light (higher temperature) and 12h dark (lower temperature). Means ± standard deviation for: seed germination G (%); (GR) mean germination rate and synchronization index (SI). Means followed by the same letter are not significant at 5% level.

Temperatures (°C)	G (%)	(GR)	(SI)
30.0-20.0	97.4 ± 0.01^{c}	0.056 ± 0.0007^{a}	2.05 ± 0.16^{ac}
25.0-20.0	95.7 ± 0.01^{a}	0.094 ± 0.0017^{b}	2.43 ± 0.16^{bc}
22.5-20.0	94.9 ± 0.01^{ab}	0.084 ± 0.0008^{c}	2.32 ± 0.10^{ab}
35.0-20.0	0	-	-
30.0-15.0	86.9 ± 0.06^{b}	0.081 ± 0.0012^{d}	2.41 ± 0.20^{d}

Judging from the results obtained for the synchronization index under 12 h-photoperiod, germination was more synchronized and differed statistically from the values obtained under all other photoperiods. There was no significant difference among the photoperiods of 2, 8 and 10 h, as well as among the photoperiods of 2, 4, 6, 8 and 10 h (Table 4). Garwood (1989) pointed out that under natural conditions, non-synchronized germination might give an adaptive advantage. Under this condition, the seed can be kept in the soil seed bank until favorable conditions trigger germination and, thus, the onset of the succession process. This fact can also be responsible for the biodiversity maintenance.

Table 4 - Seed germination of *Miconia theazeans* under alternating temperature 30-20 °C and different photoperiods of white light. Means ± standard deviation for: (GR) mean germination rate and synchronization index (SI). Means followed by the same letter are not significant at 5% level.

Light regimes (h)	(GR)	(SI)
0	-	-
2	0.046 ± 0.0028^{b}	3.58 ± 0.31^{bc}
4	0.050 ± 0.0009^{a}	3.93 ± 0.15^{c}
6	0.053 ± 0.0002^{d}	3.83 ± 0.08^{c}
8	0.047 ± 0.0014^{b}	3.62 ± 0.29^{bc}
10	0.050 ± 0.0011^{a}	3.66 ± 0.11^{bc}
12	0.056 ± 0.0007^{c}	2.05 ± 0.16^{a}

The results indicated that light was the main factor that drived the germination of the seeds of *M. theazeans*, since in no other experimental conditions germination was observed under dark conditions. Overall, under light conditions, the seeds from this species showed higher percentage of germination under both the constant (ca. 87%), and under alternating temperatures (ca. 90%). Interaction between alternating temperature and light indicated that seeds of *M. theaezans* germinated in canopy free areas.

In summary, the seeds of *M. theazeans,* germinated better under experimental conditions that resembled those naturally found in canopy gaps and open areas with broad range of alternating temperature and long photoperiods. Thus, *M. theaezans*, in natural forest, was important for colonizing large gaps and other disturbed areas due to the germination characteristics presented in the present work. Since the germination of *M. theaezans* seeds occurred only under light, the seeds probably are maintained in the soil as seed bank, thus, maintaining the biodiversity of the forest. Summing these facts to the dispersion of seeds made by birds (Knobel, 1995) *M. theaezans* could be a good species with early sucessional characteristics in reforestation projects.

RESUMO

O efeito da luz e da temperatura na germinação de sementes de *Miconia theazeans* foi analisado através de incubações isotérmicas e de alternâncias de temperaturas. Através das porcentagens finais e velocidade de germinação concluímos que a temperatura ótima de germinação localizaram-se entre 27,5 e 30 °C e pela cinética de germinação verificamos que entre 19,5 e 30 °C a germinação é dependente de processos de difusão. As temperaturas mínima e máxima foram de 12,5-15 °C e 32,5-35 °C, respectivamente. As sementes apresentaram forte dependência da presença de luz branca para a indução da germinação com a necessidade de 4-6 horas de luz diária para a máxima indução do processo. Entretanto, com a alternância de temperaturas de 30 e 20 °C, fotoperíodo de 2 horas foi suficiente para a indução da germinação sendo o máximo de indução obtida a partir de 4 horas diárias. Estes resultados indicam que *Miconia theazeans* é uma espécie importante que coloniza clareiras e áreas perturbadas em uma floresta natural.

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