Effect of light and temperature on seed germination in *Tibouchina mutabilis* (Vell.) Cogn. (Melastomataceae)

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Abstract: The effect of light and temperature on *Tibouchina mutabilis* seed germination was analyzed by isothermic incubations in the range of 10 to 40 °C, with 5 °C intervals under both continuous white light (32.85 $\mu molm^{-2}s^{-1}$) and darkness and alternating temperatures (15-20; 15-25; 15-30; 15-35; 20-25; 20-30; 20-35; 25-30; 25-35 and 30-35 °C) under both photoperiod of 12 hours and continuous darkness. The seeds of *T. mutabilis* need light to trigger the germination and no germination was observed in darkness. The range of optimum temperatures for germination was between 25 to 30 °C and the 20-25 °C alternating temperatures. These results indicate that *T. mutabilis* behaves as a pioneer species and daily alternating temperatures did not change the light sensitivity of seeds.
Keywords: *light, phytochrome, seed germination, Tibouchina.*

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Resumo: A influência da luz e da temperatura na germinação de sementes de *Tibouchina mutabilis* foi avaliada sob incubações isotérmicas de 10 a 40 °C sob luz branca contínua (32,85 $\mu molm^{-2}s^{-1}$), com intervalos de 5 °C e temperaturas alternadas (15-20; 15-25; 15-30; 15-35; 20-25; 20-30; 20-35; 25-30; 25-35 e 30-35 °C) sob fotoperíodo de 12 horas de luz branca. As sementes de *T. mutabilis* requerem luz para germinar, sendo nula a germinação no escuro em todas as temperaturas testadas. A faixa ótima de temperatura para a germinação foi de 25 a 30 °C e a alternância de temperaturas de 20-25 °C. Estes resultados indicam que *T. mutabilis* apresenta comportamento de uma espécie pioneira e que as alternâncias diárias de temperatura não alteraram a sensibilidade da semente à luz.
Palavras-chave: *fitocromo, germinação de sementes, luz, Tibouchina.*

Introduction

Melastomataceae comprises around 155 genera and about 4,500 species in whole world. It is the largest family inside of Myrtales with 1,000 species in tropical Asia, 240 in Africa, 225 in Madagascar, 50 in India and seven in tropical Australia. In tropical America, there are 100 genera and about 3.000 species. Melastomataceae centers of diversity are the Andes, Guayana, and the Brazilian coastal rain forest (Renner 2004). In Brazil, Melastomataceae is the sixth largest family of Angiosperms with about 68 genera and approximately 1.500 species. It occurs from Amazônia until Rio Grande do Sul, in almost all physiognomy with several number of species. The species of this family show large diversity of habit: shrubs, herbs, and more commonly trees and rarely as climbing plants and epiphytes. Those characteristics give wide distribution of the species in the environment (Romero & Martins 2002). Species of Melastomataceae are found in dry to moist habitats from sea level to high altitude. Species of the family are predominantly understory shrubs and herbs in Tropical Montane Forest, although few genera have radiated into lowland forest and many occur in seasonally inundated grasslands (Renner 2004). The genus *Tibouchina* occurs mainly in tropical and subtropical America with approximately 350 species (Peralta 2002).

In natural environment, the family distribution can be associated to variations in the habitat, such as canopy alteration and consequently the water availability and temperature variations. Those variations are determinant factors for the reproduction and establishment of plants. Thus, the seed germination is the first step for plants to explore news habitats which can influence the population and diversity of the species in the occupation area (Ramírez-Padilla & Valverde 2005). To another species, this variation in the environment can not influence the germination process, but the seedlings survival (Rimer & Mccue 2005). The high sensitivity of seeds to the environmental conditions results in the high probability of germination where their seedling survival is high (Ramírez-Padilla & Valverde 2005).

The specific requirement for seed germination can be associated to the life form of each species, to the environment where the plant will be established (Flores & Briones 2001, Simão et al. 2007b) and also the geographic distribution or phylogenetic origin of species (Benitez-Rodriguez et al. 2004) and still the seed characteristics (Rojas-Aréchiga et al. 2001) as its life history and development and maturation conditions (Ranieri et al. 2003)

According to Pearson et al. (2003) small seeds, usually, respond positively to the light fluences, while large seeds usually respond positively to temperature fluctuations. Photoblastic responses usually are related to the habit of the plant (Vázquez-Yanes & Orozco-Segovia 1990). The light sensitivity by seeds is dependent on phytochrome action and this sensitivity changes with the temperature (Smith 1975). In some species, alternating temperatures can overcome light requirement for germination as reported by Godoi & Takaki (2004) in *Cecropia hololeuca* and Sugahara & Takaki (2004) in *Psidium guajava*.

The main factors that influence the seed germination of tropical species are temperature, light and humidity (Barrera & Nobel 2003), which constantly are affected when gaps are formed in the canopy of the forest. (Vázquez-Yanes & Orozco-Segovia 1984, Raich 1990). Those changes induced by formation of canopy openings are fundamental to the germination and establishment of early sucessional species from soil seed bank. The tropical forest soil seed bank is involved in the establishment of wood species after natural or antropic disturbance (Tabarelli & Mantovani 1999). The authors reported the importance of Melastomataceae species in the Montane Atlantic Forest soil seed bank, including *Tibouchina mutabilis*. *T. mutabilis* is a feature species of Serra do Mar, and occurs from Rio de Janeiro to Santa Catarina, in Brazilian Atlantic Forest. This species is found mainly in secondary forest sometimes consisting in the dominant specie (Lorenzi 1992).

Melastomataceae species, mainly the genus *Tibouchina*, have potential to establishment of disturbed areas and for landscaping and street arborization. Some species of *Tibouchina* have capacity to develop in mining disturbed areas, such as *T. stenocarpa* (Lorenzo et al. 1994), and in polluted environments, such as *T. pulchra* (Mazzoni-Viveiros & Trufem 2004).

The aim of the present work was to give informations for undestanting the colonization strategies of *Tibouchina mutabilis* (Vell.) Cogn. by analysis of temperature effects on the seed germination with isothermic and alternating temperatures effects with interaction with light on seed germination are described.

Material and Methods

Tibouchina mutabilis (Vell.) Cogn. seeds were harvested in Rio Claro city gardens, Brazil, in August 2004. The seeds were dried at 25 °C in a temperature controlled room and then separated with the aid of a sieve (sieve with 0.35 cm diameter) to remove the impurity and morphologically malformed seeds. After this process the seeds were stored at 10 °C in a sealed glass bottle.

For germination experiments, approximately fifty seeds were put on two layers of water imbibed filter paper inside each of four 5 cm diameter Petri dishes were used throughout. For white light treatment, the Petri dishes were put inside colorless plastic boxes (10 x10 mm gerboxes) under day-light fluorescent lamps and dark treatment inside black gerboxes. Isothermic incubations were carried out inside germinators in the range of 10 to 45 °C with 5 °C intervals, under both continuous white light and darkness and alternating temperatures (15-20; 15-25; 15-30; 15-35; 20-25; 20-30; 20-35; 25-30; 25-35 and 30-35 °C) with 12 hours white light photoperiod or continuous darkness. White light was obtained with the aid of two 20 W day-light fluorescent lamps with photon flux density of 32.85 $\mu molm^{-1}s^{-1}$ at seed level. Seeds with at least 1 mm long roots were considered as germinated. The germinated seeds were daily scored and the dark incubated seeds were scored under dim green safe light (Amaral-Baroli & Takaki 2001). At the end of all experiments the number of not germinated seeds, the number of seeds with embryo (with the aid of magnifying glass) and the germination percentage were determined (Simão et al. 2007a). Relative frequency of germination (RF = ni:Nt, where ni is the number of germinated seeds between times ti–1 and ti); germination rate (GR = 1:t, where t is the mean time, calculated as $t = (\sum ni\ ti):\sum ni$) and synchronization index ($U = -RF\ \log_2 RF$, where RF is the relative frequency of germination) were calculated according to Labouriau & Osborn (1984). The data were submitted to ANOVA and Tukey test at $\alpha \leq 0.05$ (Sokal & Rohlf 1981). Results of germination percentage without normality and homogeneity were transformed in arcsine of $\sqrt{\%}$ before analysis.

Results

The batch of seeds used in this work presented 20 to 40% with embryo. *T. mutabilis* seeds germinated in a wide range of temperature from 15 to 35 °C however highest percentage of germination occurred in the range of 25 to 30 °C (76.7 and 61.4% of germination, respectively) which differed significantly from other tested temperatures ($p < 0.05$ by Tukey test). The minimum temperature of germination is between 10 to 15 °C and the maximum between 35 to 40 °C under continuous white light (Figure 1a and b). The germination rate did not show significant differences ($p < 0.05$) among the range of temperature of 20 to 35 °C. Although, the germination rate at 35 °C

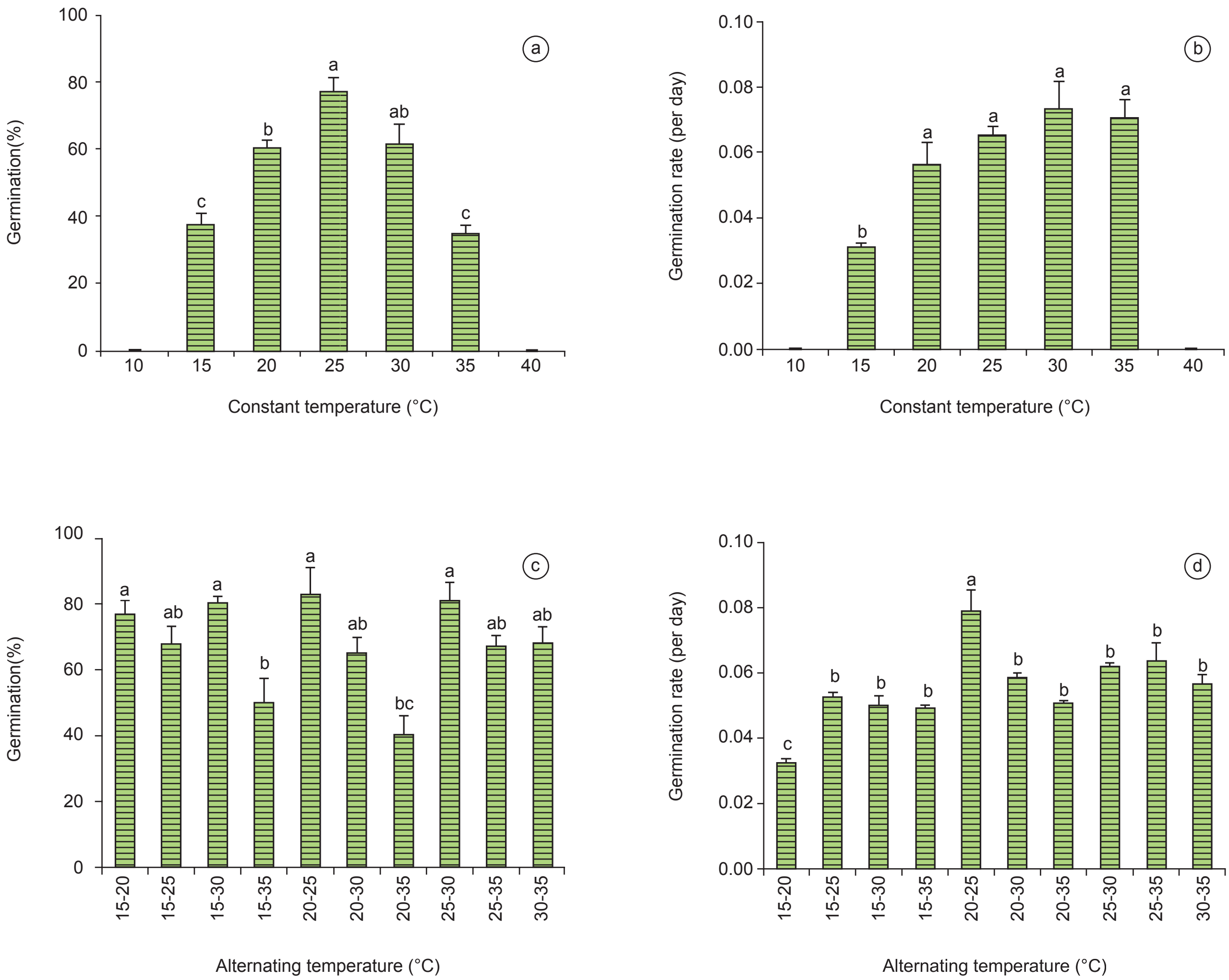

Figure 1. Percentage and rate of germination in seeds of *Tibouchina mutabilis* under isothermic incubations under continuous white light (a and b) and alternating temperatures with photoperiod of 12 hours and continuous darkness (c and d). Distinct letters indicate significant differences among treatment $p < 0.05$ by Tukey test. The bars on figures are the mean standard error.

Figura 1. Porcentagem e velocidade de germinação em sementes de *Tibouchina mutabilis* sob incubações isotérmicas e luz branca contínua (a e b) e temperaturas alternadas com fotoperíodo de 12 horas e escuro contínuo (c e d). Letras diferentes indicam diferenças significativas entre tratamentos pelo teste de Tukey $p < 0,05$. As barras sobre as figuras representam o erro padrão da média.

was not different to the optimum range of temperature, only 29.5% of seeds germinated (Figure 1b).

The seeds of *T. mutabilis* germinated in all the alternating temperature tested. The highest percentage of germination was obtained when the combination of the alternating temperature was inside the range of the optimum temperature observed at isothermic incubations (Figure 1c and d). The highest percentage germination occurred at the alternating temperatures of 20-25 °C which also presented the highest germination rate (Figure 1d) with significant differences with the other alternating temperatures ($p < 0.05$ by Tukey test). The alternating temperature with the combination of temperatures below and above the range of optimum temperatures reduced the percentage and germination rate (Figure 1c and d).

T. mutabilis seeds showed low synchronization index of germination under constant and alternating temperatures. The low synchronization index of germination indicates a wide distribution of the germination along the time (Figure 2 and 3). The events of germination occurred in peak with most seeds germinating from the seventh to nineteenth days.

Discussion

Previous studies with Melastomataceae species indicated the presence of aborted seeds (Baumgratz 1983, Zaia & Takaki 1998 and Barroso et al. 1999). This reproductive biology characteristic of those species was also reported to *T. mutabilis* (Simão et al. 2007a). The species produces large quantity of seeds being approximately 60 to 80% of them embryo less seeds. Zaia & Takaki (1998) observed that approximately 70 to 80% of seeds of *Tibouchina pulchra* and *T. granulosa* did not complete the embryo development.

Although *T. mutabilis* seeds germinated in a wide range of temperature, there was a significant reduction of germination under extreme temperatures. The reduction of germination at 35 °C, according to Andrade & Pereira (1994), Andrade (1995) and Silveira et al. (2004), seems be common to the Melastomataceae species as well

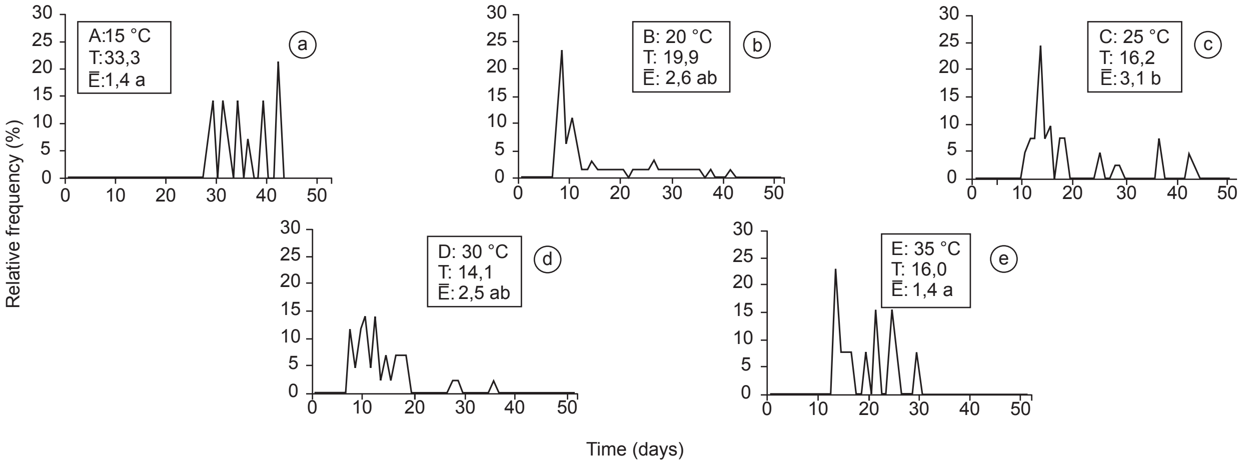

Figure 2. Relative frequency of seed germination of *Tibouchina mutabilis* under isothermic incubations. (T) Mean time ($\overline{E}$) Synchronization index of germination. Distinct letters indicates significant differences among treatments $p < 0.05$ by Tukey test.

Figura 2. Frequência relativa da germinação de sementes de *Tibouchina mutabilis* sob incubações isotérmicas. (T) Tempo médio ($\overline{E}$) Índice de sincronização da germinação. Letras diferentes indicam diferenças significativas entre tratamentos pelo teste de Tukey $p < 0,05$.

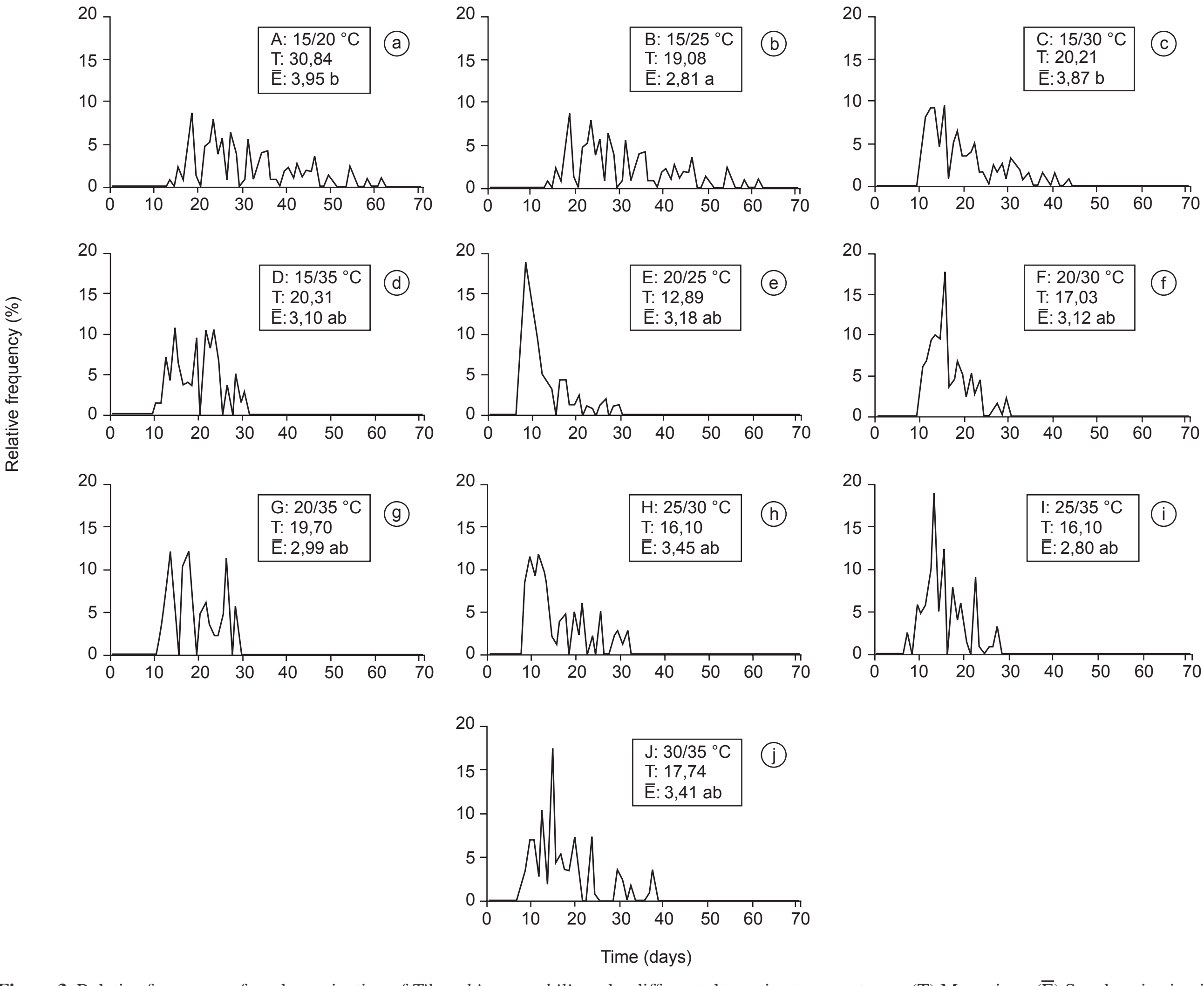

Figure 3. Relative frequency of seed germination of *Tibouchina mutabilis* under different alternating temperatures. (T) Mean time; ($\overline{E}$) Synchronization index of germination. Distinct letters indicates significant differences among treatments $p < 0.05$ by Tukey test.

Figura 3. Frequência relativa da germinação de sementes de *Tibouchina mutabilis* sob diferentes temperaturas alternadas. (T) Tempo médio ($\overline{E}$) Índice de sincronização da germinação. Letras diferentes indicam diferenças significativas entre tratamentos pelo teste de Tukey $p < 0,05$.

as the range of temperature suitable for seed germination (15 to 30 °C), as observed to *Tibouchina moricandiana* (Andrade & Pereira 1994), *Tibouchina moricandiana*, *T. benthamiana* and *T. grandifolia* (Andrade 1995), *Tibouchina pulchra* and *T. granulosa* (Zaia & Takaki 1998), *Miconia chamissois* (Válio & Scarpa 2001), *Lavoisiera cordata* and *L. francavillana* (Ranieri et al. 2003), *Miconia argentea* (Pearson et al. 2003) and *Marcetia taxifolia* (Silveira et al. 2004). Those authors also observed that seeds of those species did not germinate in darkness or present low percentage germination. Thus, we can suggest that seeds of studied Melastomataceae species have phyB (phytochrome B) controlling the germination process through Low Fluence Responses (Takaki 2001).

Some studies related that the temperature fluctuations can change the responses of seeds to light, thus seeds that germinate only under light can be able to germinate in darkness when submitted to alternating temperatures as observed by Vázquez-Yanes & Orozco-Segovia (1990) in *Cecropia obtusifolia*, Godoi & Takaki (2004) in *Cecropia hololeuca* and Sugahara & Takaki (2004) in *Psidium guajava*.

In another species the germination process is dependent on the flutuation of temperatures as observed by Válio & Scarpa (2001) in eight tropical pioneer species. In some species, small changes of temperature are enough for complete germination like as in seed of *Psidium guajava* (Sugahara & Takaki 2004), but in other species around of 10 °C range of alternating temperature is necessary (Vázquez-Yanes 1980).

The exposition of *Tibouchina mutabilis* seeds to the alternating temperatures did not restrict or increase the effect of the photoperiod on germination and in all tested temperature combinations seeds did not germinate in darkness. Similar results were also found by Válio & Scarpa (2001) in *Miconia chamissois*.

The effects of environmental factors on seed germination of tropical tree species and seedling establishment were mainly reviewed by Grubb (1977), Vázquez-Yanes & Smith (1982), Raich (1990), Vázquez-Yanes & Orozco-Segovia (1990) Orozco-Segovia et al. (1993) and Válio & Scarpa (2001). Those authors related that pioneer species require light to germinate, nevertheless for some species the seedlings did not survive under long direct sun light, delaying its development or under high temperatures due to the direct sun light those seedlings died. Those seedlings require specific conditions for seed germination and seedling establishment. In Melastomataceae these characteristics were observed in *Miconia affinis*, *Miconia gracilis* and *Miconia nervosa* by Elisson et al. (1993) and *Tibouchina herbacea* by Almasi (2000).

In *T. mutabilis* the results of germination rate, synchronization index and the relative frequency under constant and alternating temperatures indicate a wide distribution of the germination along the time. This characteristic can be responsible for the maintenance of viable seeds in the soil bank until the condition is suitable for germination and seedling establishment. The germination mean time is a good index to evaluate the rate of establishment of species in specific habitat (Ferreira et al. 2001) as well the synchronization index and the relative frequency. These responses can be influenced by environment change of the habitat (Raich 1990). To some species, these changes did not influence directly on germination response, but on the survival of seedlings (Rimer & McCue 2005).

T. mutabilis seed germination is triggered by light, indicating the presence of the phytochrome in the seeds, monitoring light of the environment. According to Andrade & Pereira (1994) the light is the main factor for induction the seed germination in Melastomataceae species. Zaia & Takaki (1998) reported that *T. pulchra* and *T. granulosa* seeds germinated when submitted to very low fluency of white light, but no germination was observed under far-red light and under continuous darkness.

The results presented here indicate that seeds of *Tibouchina mutabilis* are sensitivity to white light and respond to the environmental changes as typical pioneer species, with germination only under white light although the daily alternating temperatures did not change the light sensitivity as reported for other pioneer species. The germination under different temperatures is widely spread along the time, even though under optimum range of temperature (Figure 2 and 3). Possibly, those characteristics are responsible for the presence of *T. mutabilis* seeds in the soil seed bank as reported by Tabarelli & Mantovani (1999) in Atlantic Forest.

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