

Phytochrome and Temperature Control of Seed Germination in *Muntingia calabura* L. (Elaeocarpaceae)

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ABSTRACT

The temperature range of germination of seeds of Muntingia calabura L. was 15-35°C and the optimum temperature at 35°C where more seeds germinated at short period. The fluence response curves demonstrated the involvement of phytochrome in the control of germination through the low fluence response. The effect of photoperiod showed that the species need long daily exposures to white light and that shade light inhibited completely seed germination, confirming as pioneer species that colonized only large gaps in the forest.

Key words: Seed germination, phytochrome, light, temperature, *Muntingia calabura*

INTRODUCTION

The ecophysiological study of seeds allows the understanding of the mechanisms which regulate seed longevity in the soil, dormancy breaking, germination and plant establishment in natural conditions. This is a crucial aspect of plant biology is crucial when studying species which only became established in disturbed sites of mature vegetation (Vazquez-Yanes & Orozco-Segovia, 1982).

Light is one of the most important environmental factor regulating growth and development of plants. When absorbed by chlorophyll, light represents a source of energy. However, when absorbed by phytochrome, light controls plant photomorphogenesis. Phytochrome exists under a red (Pr) and a far-red (Pfr) absorbing distinct forms. Upon exposures to red light, Pr undergoes photochemical conversion to a physiologically active far-red absorbing form, Pfr, which triggers a variety of complex physiological responses such

as flowering and seed germination (Mancinelli, 1994).

Therefore, as pointed out by Casal and Sánchez (1998) phytochrome enable the plant to detect shading by other plants and thus induces the alteration of metabolism and development. Phytochrome is, then, extremely important for plants growing under canopies, under natural conditions. In such situations, plants experience not only a reduction in irradiance but also altered spectral light quality due to selective absorption of blue and red wavelengths by chlorophyll. In particular, the red to far-red ratio (R/FR) of incident light may be dramatically reduced under a closed canopy compared with values in sunlight, and may also vary widely. Thus, R/FR is an important cue by which plants may detect microenvironmental variation in vegetation shade, both from overstory foliage and from neighbors (Schmitt et al., 1999).

Seeds of many plant species, mostly those that inhabit open, or frequently disturbed habitats, require light to germinate. Environmentally

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induced photosensitivity of seeds is often interpreted as an adaptation ensuring that seeds will germinate in sites in which the probability of seedling establishment is high. Such a mechanism would be particularly advantageous for species with small seeds whose seedlings cannot survive in established vegetation (Gross, 1985).

Temperature is another factor which controls seed germination in the nature, and the knowledge of cardinal temperatures is important for the understanding of plant species occurrences (Labouriau & Agudo, 1987). The light requirement by seeds is also dependent on the temperature as reported by Smith (1982).

Muntingia calabura is a pioneer species which occurs from Southern Mexico to Bolivia and Brazil and it has been used in heterogeneous reforestation of disturbed areas (Campos, 1982). In the present work we analyzed the effect of light and temperature on seed germination of *M. calabura*.

MATERIAL AND METHODS

Fruits of *M. calabura* were collected from five individuals in Piracicaba and Rio Claro, São Paulo State, Brazil. Seeds were extracted from fruits by peeling them and passing through a plastic strainer and then washed in distilled water. After this procedure, these were dried at 25°C for at least 24 hours and stored in closed jar at 5°C.

Seeds were sowed in 5mm diameter Petri dishes with filter paper imbibed with distilled water. All experiments were carried out in four replicates. The number of seeds in each Petri dish was random, due to reduced seed size as proposed by Cone and Kendrick (1985) for *Arabidopsis thaliana*. Seeds were scored daily and those with at least 1mm long root were considered as germinated. At the end of the tests, the remaining not germinated seeds were counted to attain the total number of seeds in each Petri dish. Seeds incubated under darkness were scored under dim green safe light (Kendrick & Frankland, 1969). White light was obtained with the aid of 15W daylight fluorescent lamps. Far-red light was obtained with the aid of two of each red and blue cellophane papers wrapping the 25W incandescent bulbs. The light fluences and 655-665 and 725-735 nm fluences were measured with the aid of a LI-1800 spectroradiometer (LICOR, USA).

Temperatures from 15°C to 40°C were tested at 5°C intervals under continuous white light and darkness for determination of optimum temperature. Photoperiods from 0 to 24 hours were tested at 35°C under white light (25.55 μ W.m⁻²). For fluence-response curves, seeds were sowed in Petri dishes placed in plastic trays under different fluences achieved using colorless or neutral glass plates and neutral plastic mesh. Shade light effects on seed germination were tested at 35°C and the seeds were kept under white light enriched with far-red. Simulated canopy had 6.85 μ W.m⁻², and calculated photoequilibrium of phytochrome of 0.2 (Mancinelli, 1993).

Percentage, velocity and relative frequency of germination, were determined according to Labouriau and Agudo (1987) as follow: $G=(n/a) \times 100$; $V=1/t$; $t=(\sum n_i \times t_i / \sum n_i)$; $FR=n_i/N_t$, where n = total number of germinated seeds; a = total number of seeds; n_i = number of germinated seeds between two consecutive observations ($t-1$) and (t) and N_t = number of germinated seeds. The significance of $\arcsin\sqrt{\%/100}$ among treatments were obtained by Tukey test (Snedecor & Cochran, 1967).

RESULTS

Temperature strongly affected germination of *M. calabura* seeds, as shown by Figure 1 and 35°C was the best temperature for germination percentage and velocity. Accordingly, as temperature increased, germination velocity increased significantly. Figure 2 shows the polygons of relative frequency of seed germination, demonstrating that germination was more homogeneous with a well defined peak of germination at 30° and 35°C.

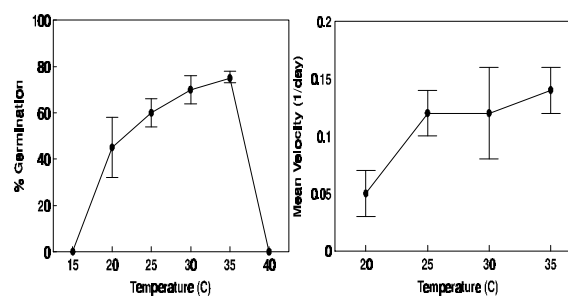


Figure 1 - Effects of temperature on germination of *M. calabura* seeds. (A) percentage of germination; (B) mean time velocity to germination.

Long photoperiod also induced germination of these seeds. (Figure 3). The best germination percentage was obtained for seeds exposed to 6 hours of light or more, while germination velocity demonstrated that at least 8 hours were necessary. After such value, light exposure does not improve nor impair mean time to germination. Mean velocity of germination also profits from light exposure, in similar way as for mean time. The figure 4 shows that germination was more uniform as of 8 hours photoperiod, with a well defined germination peak. Statistically differences are shown in the figure 4.

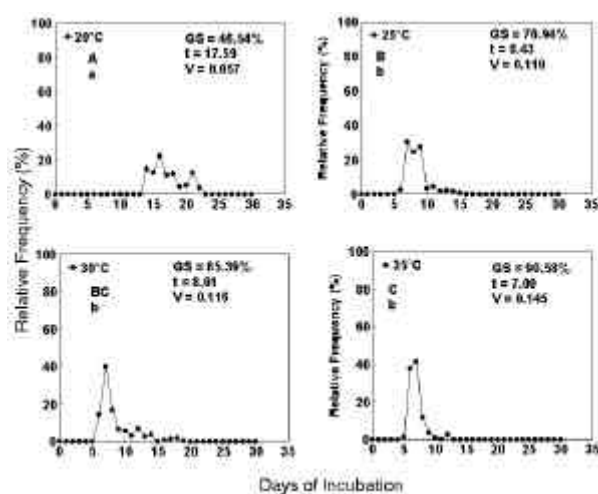


Figure 2 - Polygons of Relative Frequency to temperature effects on seed germination of *M. calabura* in light conditions. GS = % of germinated seeds; t = mean time for germination; V = mean velocity of germination. The letters show statistics differences between treatments. The capital letters refers to germination percentage and the tiny ones refer to velocity.

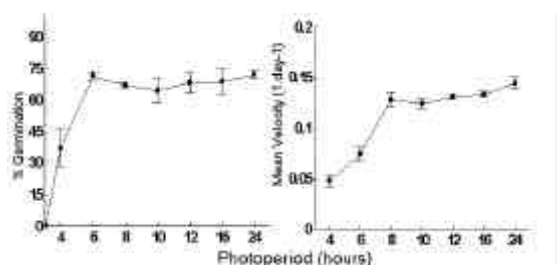


Figure 3 - Effects of photoperiod on germination of *M. calabura* seeds. (A) percentage of germination; (B) mean time velocity to germination.

The curve of fluence-response demonstrated the dependence of percentage and velocity of germination with the white light fluence (Figure 5). Figure 6 shows the polygons of relative frequency of germination, which showed that with 25.55 W.m^{-2} , germination had a well defined peak, after 7.5 days from sowing. At the other fluences, the germination of seeds was more scattered in time. No germination was observed under shade light, showing that seeds of *M. calabura* do not germinated under canopy in natural conditions.

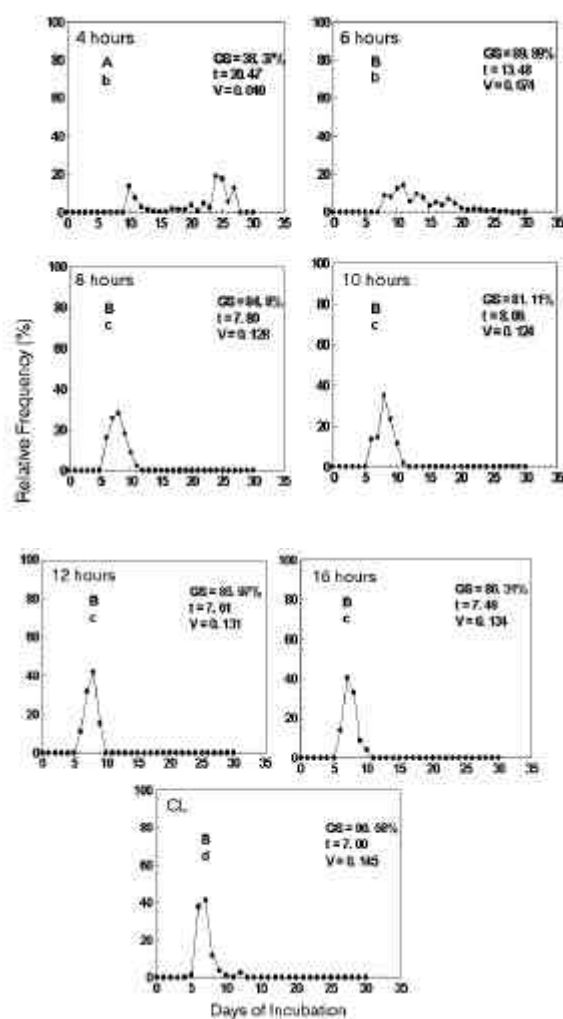


Figure 4 - Polygons of Relative Frequency to photoperiod effects on seed germination of *M. calabura* in light conditions. GS = % of germinated seeds; t = mean time for germination; V = mean velocity of germination; CL = continuous white light. The letters show statistics differences between treatments. The capital letters refers to germination percentage and the tiny ones refer to velocity.

DISCUSSION

Dependence of *M. calabura* seeds on temperature and light quantity and quality denotes an adaptive switch towards germination in open spaces, either large gaps or forest boundary. Many tropical species need high temperatures for the promotion of germination. *M. calabura* usually occur in disturbed habitats, where natural vegetation was destroyed in different proportions. In those gaps, soil surface temperatures, may be increased, depending on gap size, daily photoperiod, climate and season (Vazquez-Yanes & Smith, 1982; Swaine & Whitmore, 1988).

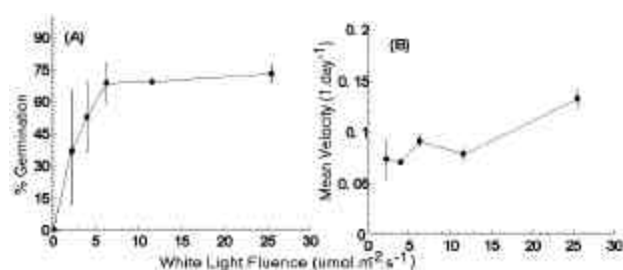


Figure 5 - Curve of fluence-response for induction of seed germination of *M. calabura*. (A) percentage of germination; (B) mean velocity to germination.

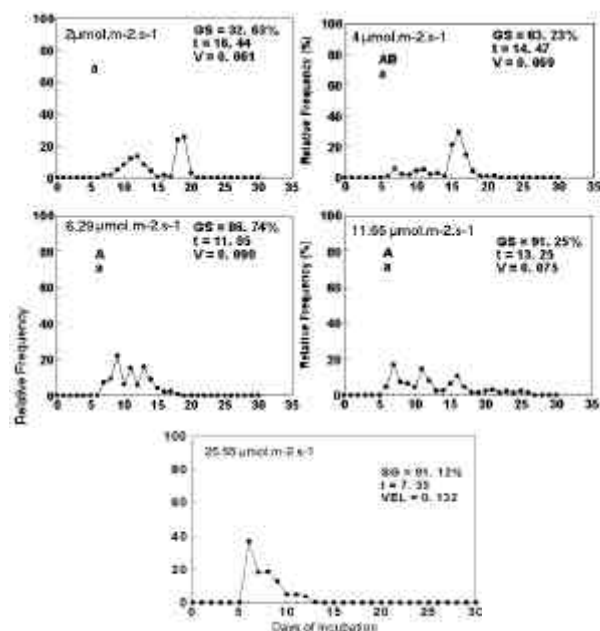


Figure 6 - Relative Frequency polygons to irradiance effects on seed germination of *M. calabura* in light conditions. GS = % of germinated seeds; t = mean time for germination; V = mean velocity for germination. The letters show statistics differences between treatments. The capital letters refers to germination % and the tiny ones refer to velocity.

Many studies have been carried out relating characteristics of tropical species to ecological groups into succession process (Budowski, 1965; Denslow, 1980; Bazzaz & Pickett, 1980; Vazquez-Yanes & Orozco-Segovia, 1984; Brokaw, 1987). Vazquez-Yanes and Smith (1982) studied the germination of two tropical pioneer species, *Cecropia obtusifolia* and *Piper auritum* and proposed that phytochrome acted detecting gap size. Both species required long simulated daily photoperiods to attain maximum germination suggesting that they occurred only in large gaps.

The effect of photoperiod indicates how plant species could colonize gap in the forest canopy by shade avoidance response and perception of proximity neighboring vegetation (Schmitt et al., 1999). Zaia and Takaki (1998) working with seeds of two pioneer species, *Tibouchina pulchra* and *T. granulosa* observed that at least 8 hours photoperiod was required for maximum induction of germination.

Our results agree with those cited above. Relating them with natural conditions, we inferred that a 8 hours minimum daily photoperiod simulated a large gap and the best temperature for germination at 35°C backing to that relation. The fluence response curves indicated that phytochrome controlled seed germination in *M. calabura*.

Seeds in their natural environment undergo shading by two manners. One is when they are buried in soil or lie beneath litter layer, and the other is when they are beneath the canopy. Both cases affect light quantity and quality: under sun light, R/FR is around 1.2 but it falls to approximately 0.55 under 3mm soil and 0.1 under dense shading (Frankland, 1981). Therefore, our results indicated that *M. calabura* seeds would not germinate beneath canopy due to the low irradiances and R/FR ratio, typical of such places.

These responses agreed with Cone and Kendrick (1986), who studied about one of phytochrome function in natural environment. According to these authors, phytochrome acts as transducer, allowing plants to growth and develop under favorable conditions. The R/FR reversibility is very important, as it allows shading detection by canopy leaves. As an adaptation strategy to the environment, Smith (1986) cited that in both shade-tolerant and shade-intolerant species, shading perception was the prior requirement to the establishment of the species. It seemed clear that shade-avoiding reactions are phytochrome mediated by R/FR perception. Beneath canopy,

R/FR underwent a reduction, which was proportional to canopy density and age (Smith, 1982).

M. calabura has an abundant and long fructification, producing small sweet fruits with plenty of seeds. These fruits are dispersed by bird and bat. The seeds are light dependent for germination and the seedlings will certainly undergo light competition under canopy. The 6 days minimum period to start germination can be considered short compared to other species. This species shows a retardation in germination when exposed to low irradiances and has no germination under shade-light ($\phi=0.2$), rich in far-red light. Seed longevity seems to be high, at least when stored at low temperature. The experiments were carried out for 9 months and no alteration on germination of *M. calabura* seeds was noticed during this period.

RESUMO

As sementes de *Muntingia calabura* L. germinam nas temperaturas de 15 a 35C sendo a temperatura ótima de 35C onde maior número de sementes germinam em menor período. A curva de fluência-resposta demonstra que o fitocromo controla a germinação de sementes através da resposta de fluência baixa. A resposta da semente ao fotoperíodo e à completa inibição pela luz de sombreamento indicam que a espécie coloniza somente clareiras grandes de florestas, confirmando a espécie como pioneira.

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