Germination and Emergence of Trumpet Flower (*Tecoma stans*) under Different Environmental Conditions[1]

Germinação e Emergência de Amarelinho (Tecoma stans) sob Efeito de Diferentes Condições Ambientais

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ABSTRACT - The aim of this work was to analyze the effect of temperature and light intensity on trumpet flower seed germination, as well as the effect of seeding depth on its emergence. To study the influence of temperature, nine temperature intervals were evaluated, ranging from 15.0 to 40.0 °C. A randomized block design experiment was used with five replications and 20 seeds per replication, and performed twice. To evaluate light intensity on seed germination, a randomized experimental design was used with eight replications and 25 seeds per replication. The treatments applied were: photoperiod with temperature alternation; photoperiod with constant temperature; darkness with temperature alternation; and darkness with constant temperature. The photoperiod consisted of 8 hours of light and 16 hours of darkness, and the constant temperature was 25 °C. The treatments with temperature alternations were established with 8 hours at 30 °C, and 16 hours at 20 °C. Germination was assessed daily to calculate the total percentage of germination as well as the Germination Velocity Index (GVI). To study the influence of seeding depth on plant emergence, 25 seeds were seeded at 0, 20, 40, and 80 mm in pots with sieved soil. The experiment was arranged in a randomized block design with four replications. Seedling emergence was monitored daily until the 15th day after seeding. After that period, the total percentage of emergence was calculated for each experimental unit, as well as the Emergence Velocity Index (EVI). Formation of normal seedlings and the Germination Velocity Index were different among temperatures and higher germination percentages were observed between 20.3 °C and 37.5 °C. Tecoma stans seedlings did not germinate when planted at 40 and 80 mm depth. However, the seedlings placed on the soil surface had an emergence percentage of 72. At 20 mm depth, the emergence rate was 31%.

Keywords: temperature, light, photoperiod, photoblastism.

RESUMO - Objetivou-se com este trabalho avaliar o efeito da temperatura e luminosidade na germinação, bem como o efeito da profundidade de semeadura na emergência do amarelinho. Nos estudos de influência da temperatura na germinação foram avaliados nove intervalos de temperatura entre 15 °C e 40 °C. Foi utilizado o delineamento inteiramente casualizado, com cinco repetições e 20 sementes por repetição. A influência da luminosidade foi avaliada com os tratamentos: fotoperíodo com alternância de temperatura, fotoperíodo com temperatura constante, escuro com alternância de temperatura e escuro com temperatura constante. Em ambos os estudos foram calculados a porcentagem e o índice de velocidade de germinação (GSI). A influência da profundidade de semeadura foi avaliada depositando-se 25 sementes a 0, 20, 40 e 80 mm de profundidade, em vasos contendo solo peneirado. O delineamento experimental foi o de blocos ao acaso com quatro repetições. Foram calculados a porcentagem e o índice de velocidade de emergência (ESI). Os intervalos de temperatura testados não influenciaram a protrusão da raiz primária, e a faixa de temperatura ótima para formação de plântulas está entre 26,4 °C e 37,5 °C. As sementes são fotoblásticas neutras, e a alternância de temperatura não favorece a germinação da espécie. Plântulas de amarelinho não emergiram quando

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as sementes foram depositadas nas profundidades de 40 e 80 mm, ao passo que sementes posicionadas na superfície do solo apresentaram porcentagem de emergência de 72%; na profundidade de 20 mm a emergência foi de 31%.

Palavras-chave: temperatura, luz, fotoperíodo, fotoblastismo.

INTRODUCTION

Basic studies on weed biology are scarce – particularly on those that infest areas of cultivated pastures. Understanding the behavior of the germination of weed species linking it to environmental factors plays an important role in interpreting the ecological behavior of the species in the field and enables the development of strategies to reduce the potential of the seed bank and thus the decrease in supplying new individuals of undesirable plants (Souza Filho, 1998).

The depth of the soil in which a plant is able to germinate and produce a seedling varies among species and presents ecological and agronomic importance. Greater seedling emergence of coatbuttons when the seeds were deposited on the surface and were partially buried was observed; when deposited at depths greater than 1 cm, there was a reduction in emergence (Guimarães et al, 2002.). Labonia et al. (2009) observed greater emergence of five species of the family Convolvulaceae (morning glory) when seeds were deposited on the soil surface.

Many weed species germinate only when arranged in small soil depths because these seeds, in most cases, require incident light to start the germination process, being called positive photoblastic. According to Benvenuti (1995), as soil depth increases, the light is strongly attenuated; typically seeds of these species when deposited at greater depths are unable to emerge. The temperature also varies with the depth of the soil; in the vicinity of the surface it is very similar and was significantly attenuated after only 5 cm depth (Gasparim, 2005).

The germinated seed can be considered in two ways. From the viewpoint of plant physiology, the radicle protrusion indicates the end of germination and the subsequent development is considered post-germination. However, the concept of germination in seed technology involves the development of embryonic structures and the formation of a seedling in which all its contributing parts are evident (Brasil, 1992; Marcos Filho, 2005).

In connection with the germination of weed species described in literature, there are works that use both the primary root protrusion (Carvalho & Christoffoleti, 2007; Socolowski et al., 2008) and the formation of normal seedlings (Wang et al. 2009). Thus, there is no consensus about the best criterion to be adopted and the results are comparable.

The *Tecoma stans* species, popularly known as trumpet flower, was introduced in Brazil as ornamental and became later an important weed in cultivated pastures and abandoned areas in the country (Lorenzi, 2008). This species produces lots of seeds with membranous wing (Renó et al., 2007), which are easily spread by wind. This plant also spreads vegetatively, by pieces of stem and root.

Aiming to understand the distribution and establishment of the species and provide information for integrated management programs, the effects of temperature and light on germination and the effects of sowing depth on trumpet flower seedling emergence were evaluated.

MATERIAL AND METHODS

The research was conducted between the months of November 2011 and August 2012. The seeds were collected in August 2011 in a natural infestation area in cultivated pastures of Escola Superior de Agricultura "Luiz de Queiroz"- USP, Piracicaba, São Paulo. The seeds were previously subjected to germination tests in order to verify the feasibility. All seeds were stored in a kraft paper bag, in a dry chamber at 20 ^oC and 50% RH. The studies that evaluated the temperature and light were performed at the Laboratory of Seed Analysis of Escola Superior de Agricultura "Luiz de Queiroz"- USP, Piracicaba, São Paulo.

Temperature

To determine the effect of temperature, 20 seeds were distributed on three sheets of blotting paper on a Petri dish (diameter 8 cm); the amount of water applied corresponded to 2.3 times the weight of the paper. The Petri dishes were incubated at temperatures ranging from 15 °C to 40 °C (15.1 at 17 °C; 17.5 at 19.6 °C, 20.3 at 22.2 °C, 23.6 at 25.6 °C, 26.4 at 28.5 °C; 28.8 at 30.9 °C, 31.7 at 33.9 °C, 34.5 at 37.5 °C, 37.9 to 40.9 °C) in thermogradient table (Van den Berg brand, model 890) under a photoperiod of eight hours. A completely randomized design with five replications was used, and the experiment was repeated twice.

Light and alternating temperature

To evaluate the effect of light, 25 seeds were placed in transparent plastic boxes (0.11 x 0.11 x 0.03 m), with two sheets of blotting paper (0.105 x 0.105 m) moistened with amount of water equivalent to 2.5 times the weight of the paper. The following treatments were evaluated: photoperiod of eight hours a day and alternating temperature (8h/30 °C and 16h/20 °C); photoperiod of eight hours a day at a constant temperature (25 °C); continuous darkness and alternating temperature (8h/30 °C and 16h/20 °C); and continuous dark at a constant temperature (25 °C). The experimental design used was completely randomized with eight replications. Evaluation of germination of seeds stored in the dark was taken in a dark room under green light because its wavelength does not stimulate seed germination.

Sowing depth

The research was conducted in a greenhouse; during its conduction, the average daily temperature was 29 °C, with maximum of 36 °C and minimum of 19.5 °C.

The experimental units consisted of plastic pots with 190 mm diameter, 150 mm height and capacity for 2.8 liters filled with soil (Eutroferric Red Nitosol) of clayey texture (34% sand, 13% silt and 53% clay). The bottom of the vessel was sealed with filter paper, and soil moisture was monitored gravimetrically, so as to keep a level close to 80% of the field capacity. Sowing was done by depositing 25 seeds per pot at depths of 0, 20, 40 and 80 mm, and covering them with soil to a pre-defined height. The experimental design used was randomized blocks with four replications.

Forms of evaluation and statistical analysis

Evaluations of the effect of light and temperature on germination were performed daily according to primary root protrusion and the production of normal seedlings. The primary root protrusion was considered when it had 2 mm or more in length (Carvalho & Christoffoleti, 2007). As a normal seedling, were considered those that had the essential structures of the embryo developed (Brasil, 1992).

Evaluations of the effect of sowing depth were performed within 15 days after the experiment was established, and the seedling was considered emerged after the expansion of the cotyledons. With the data, we calculated the germination or emergence speed index (GSI/ESI) and percentage, according to Maguire (1962).

The evaluation of the effect of temperature was performed twice, and the data were analyzed together. On the GSI and germination percentage (normal seedling formation) data, transformations were employed (Box & Cox, 1964). These data were also used in the germination percentage (normal seedling formation) and GSI (primary root protrusion), when assessing the effect of light.

The nonparametric test based on orders (Conover, 1981) was used in germination percentage data, considering the primary root protrusion in both tests performed in laboratories. Then, an analysis of variance with application of the F test was made; if significant, the Tukey test at 5% probability was performed. Furthermore, analysis of the temperature ranges was conducted with the use of regression equations. For this, quadratic polynomial equations were fitted to the average temperature intervals of data (Brancalion et al., 2008; Wang et al., 2009.).

RESULTS AND DISCUSSION

Temperature

The temperatures did not influence the germination percentage when evaluating primary root protrusion (p> 0.05, Table 1). The species *Tabebuia rosea*, another Bignoniaceae, showed a similar behavior, showing no difference in germination percentages between the temperatures of 15 °C and 40 °C (Socolowski & Takaki, 2007). Socolowski et al. (2008) found no difference in the percentage of germination of trumpet flower seeds between temperatures of 15-40 °C.

The temperatures studied influenced the formation of normal seedlings (p<0.05, Table 1). In the intervals of 15.1-17.0 °C and 37.9-40.9 °C the percentages of normal seedling formation decreased, presenting 58 and 75%, respectively (Figure 1). Seeds of *Urena lobata*, an important weed in pastures, showed similar behavior, where lower percentages of seedlings formed at temperatures of 15 and 40 °C were observed (Wang et al., 2009).

In cold conditions the metabolic activity is relatively low, which decreases the germination percentage and rate (Okuzanya, 1980). Moreover, high temperatures for long periods may induce protein denaturation. Generally, species with wide distribution feature germination in a wide temperature range (Larcher, 2000).

Unlike the germination percentage, germination speed index (GSI), evaluating the primary root protrusion, were lower as the temperature ranges studied decreased. The same occurred when evaluating the formation of normal seedlings (Figure 1). Socolowski & Takaki (2007), in research with seeds of *T. rosea*, saw no difference between the germination rate means, when measured at temperatures of 20, 25, 30 and 40 °C.

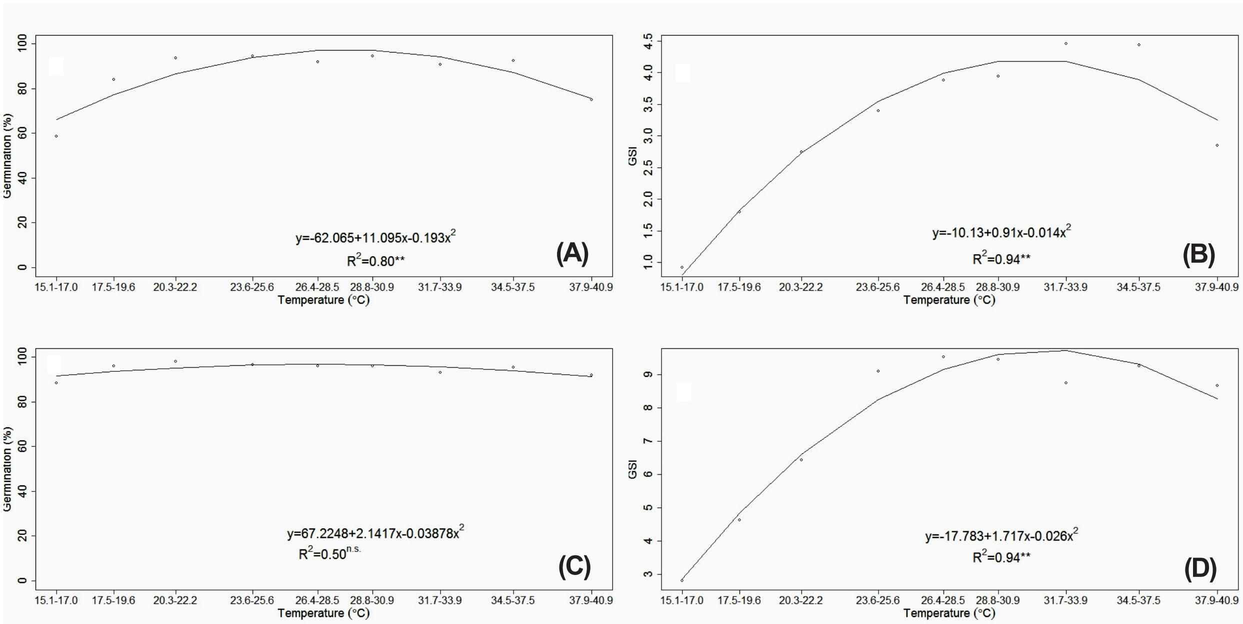
According to the normal seedlings and GSI formation percentage results, the optimum temperature for germination of trumpet flower is in the range of 26.4 °C to 37.5 °C (Table 1). According to Marcos Filho (2005), the optimum temperature for germination may be that in which occurs the most efficient combination of percentage and speed of germination, that is, the maximum germination in the shortest period of time.

As expected, the rate of primary protrusion was higher than that of normal seedlings production, since the primary root is usually the first portion of the seedling developing from germination. The differences in germination percentage are due to not accounting for abnormal seedlings when evaluating the primary root protrusion, resulting in higher values of germination, compared with the production of normal seedlings. Therefore, the issue of primary roots was less affected by temperature when compared with normal seedling formation. This indicates that the formation of shoots of trumpet flower seedlings is more sensitive to temperature changes than the root system and that there are differences in the temperature requirement for the development of different parts of the seedling.

Table 1 - Germination (G%) and germination speed index (GSI) of *Tecoma stans* seed in thermogradient table between the temperatures of 15 and 40 °C, in accordance with the production of normal seedlings (PN) and primary root protrusion (PRP). Piracicaba, SP

Temperature (°C)	PN		PRP	
	%G[1/]	IVG[2/]	%G[3/]	GSI
15.1 - 17.0	58.5 c	0.92 e	86.0	2.82 d
17.5 - 19.6	84.0 ab	1.79 d	97.0	4.62 c
20.3 - 22.2	93.5 a	2.74 c	97.0	6.44 b
23.6 - 25.6	94.5 a	3.39 bc	94.0	9.04 a
26.4 - 28.5	92.0 a	3.88 ab	95.0	9.51 a
28.8 - 30.9	94.5 a	3.94 ab	97.0	9.45 a
31.7 - 33.9	90.7 a	4.45 a	96.0	8.74 a
34.5 - 37.5	92.5 a	4.43 a	96.0	9.25 a
37.9 - 40.9	75.0 bc	2.84 c	91.0	8.66 a
Mean	86.13	3.16	94.33	7.62
F	14.88**	69.78**	1.48^{ns}	148.16**
CV (%)	24.58	8.43	6.93	8.31

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. [1/] Data transformed by (x^3); [2/] data transformed by (“x); [3/] analysis of variance based on orders; ** F value significant at 1% probability; NS value of F not significant at 5% probability.



(A) percentage of germination - PN; (B) germination speed index - PN; (C) percentage of germination - PRP; (D) germination speed index - PRP. **value of F significant at 1% probability; NS value of F not significant at 5% probability; Piracicaba, São Paulo.

Figure 1 - Germination (G%) and germination speed index (GSI) of *Tecoma stans* seed in thermogradient table between the temperatures of 15 and 40 °C, in accordance with the production of normal seedlings (PN) and primary root protrusion (PRP).

The adjusted regressions showed highly significant R^2, as well as the equation coefficients, for the of normal seedlings germination percentage and the normal seedling germination speed index and primary root protrusion (Figure 1). In the case of regression adjusted for the percentage of germination of the primary root protrusion, the R^2 was low (0.5) and not significant (Figure 1). In this particular case, other equations were tested, but all had the same behavior.

Light and alternating temperature

Although trumpet flower infest areas where shading usually does not occur, such as pastures and abandoned areas, no influence of light was observed to initiate germination, primary root protrusion occurring in the presence and absence of light, which characterizes these seeds as neutral photoblastic. Socolowski et al. (2008) also observed the primary root protrusion of trumpet flower seeds in the presence and absence of light.

Although the trumpet flower seeds germinate in the dark (primary root protrusion), the percentage of normal seedlings of tests conducted in the dark was lower than that of tests driven with light (Table 2). These results indicate that the absence of light may impair the formation of seedlings of this species. Steinbauer & Grigsby (1957) claim that although most seeds

Table 2 - Germination (G%) and germination speed index (GSI) of *Tecoma stans* seeds maintained in the presence and absence of light and temperature being constant (25 °C) and alternating day/night (20-30 °C), according to the production of normal seedlings (PN) and primary root protrusion (PRP). Piracicaba, São Paulo

Light and temperature regime	PN		PRP	
	G%[1/]	GSI	G%[2/]	IVG[1/]
Photop./25 °C	93.50 a	3.56 a	95.00	11.73
Photop./20-30 °C	89.00 a	2.88 b	95.50	11.50
Dark/25 °C	36.50 b	0.93 c	93.00	11.37
Dark/20-30 °C	31.50 b	0.63 c	96.50	11.25
Mean	62.63	1.99	95.00	11.47
F	330.91**	175.24**	0.87^{ns}	0.62^{ns}
CV %	14.58	15.39	4.69	12.96

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. [1/] Data transformed by (x^2); [2/] analysis of variance based on orders; ** F value significant at 1% probability; ^{ns} value of F not significant at 5% probability.

germinate both in light and in the dark, some will germinate less or not completely under totally dark conditions.

In the treatments conducted in the dark, some seeds that germinated in the dark originated seedlings whose cotyledons remained within the integument and had stunted hypocotyl. According to Brasil (1992), in order for a seedling to be classified as normal, 50% or more of the cotyledon must be intact, with no evidence of damage or deterioration of the shoot apex; therefore, seedlings where cotyledons remained within the integument were not counted.

Brancalion et al. (2008) obtained similar results, for although seeds of *Heliocarpus popayanensis* emit primary root in the absence of light, the percentage of normal seedlings was lower in tests conducted in the dark, when compared with those conducted with light. Wang et al. (2009) found no difference in seedling rate of *U. lobata* when seeds were germinated in light and dark.

Socolowski et al. (2008) observed a higher percentage of trumpet flower seedling emergence in a sunny environment compared to a shady the environment; in addition, it was observed that, in a shade environment, only 1.5% of the plants had survived 30 days after sowing, the whereas, in full sun, 96.5% of the seedlings survived, confirming the invasive nature of this species.

Regarding the germination speed index, there was no difference between treatments when primary root protrusion was evaluated. When considering normal seedling, the highest germination speed was observed in the treatment with constant temperature and photoperiod (Table 2).

Alternating temperature did not favor germination (PRP) and trumpet flower normal seedling. It can be observed (Table 2) that there was no difference in the percentage of normal seedlings between treatments conducted in light and dark. Carvalho & Christoffoleti (2007) observed that the condition of alternating temperature improved the germination of the *Amaranthus* species. Mondo et al. (2010) also observed more favorable results for percentage and speed of germination under alternating temperature seed *Digitaria bicornis.*

Sowing depth

Highest percentage and highest speed of emergence were observed when seeds were deposited on the soil surface (Table 3). No emergence was observed when seeds were deposited at 40 and 80 mm of soil depth. Wang et al. (2009) found a higher percentage of emergence when seeds of *U. lobata* were deposited on the surface, while the rate of seedling emergence reduced 10% per cm deep in the soil.

In deeper layers of the soil, light and seed size are usually limiting factors to emergence (Wang et al., 2009). In fact, the results presented (Table 2) show that trumpet flower is able to germinate in the dark; so the seeds of the species deposited at depths greater than 2 cm probably germinated, but the length of hypocotyl was not sufficient for seedlings to reach the soil surface. The lack of adequate energy reserves of the seed or its small size has been suggested in other studies as a possible cause of the failure on the seedling emergence in large planting depths (Dias-Filho, 1998).

Souza Filho et al. (1998) observed that the species *Cassia tora* emerged at 8 cm, while *U. lobata* did not. These authors attributed this behavior to the smaller size of the seed of *U. lobata*. In contrast, Toledo et al. (1993)

Table 2 - Germination (G%) and germination speed index (GSI) of *Tecoma stans* seeds maintained in the presence and absence of light and temperature being constant (25 °C) and alternating day/night (20-30 °C), according to the production of normal seedlings (PN) and primary root protrusion (PRP). Piracicaba, São Paulo

Depth (mm)	% E	ESI
0	72a	2.53a
20	31b	0.81b
40 1/	0	0
80 1/	0	0
Mean	51.5	1.67
F	47.13**	875.83**
CV %	16	4.92

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. 1/ Data transformed by (x^2); 2/ analysis of variance based on orders; ** F value significant at 1% probability; ns value of F not significant at 5% probability.

observed that seedling emergence of *Xanthium strumarium* occurred almost continuously up to 8 inches deep. However, the emergence of seedlings when seeds were in depth of over 8 cm was significantly lower. Oxygen concentration decreased with sowing depth (Benvenuti et al., 2001). According to Marcos Filho (2005), low concentrations of oxygen in these conditions can paralyze the germination of many seeds, which may also explain the lack of germination of the species at greater depths.

Trumpet flower seeds can germinate in different light conditions and in a wide range of temperature, with the formation of normal seedlings above 50% even at temperatures below 20 °C and above 37 °C. However, sowing depth is a limiting factor for the emergence, so the inversion of soil with cultivation could be a way to control the species. However, this type of operation is not feasible or frequently performed in pastures, which explains the ability of the species to germinate and establish in them.

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