

# Temperature effect in the number of seedlings per seed in cultivated specimens of *Handroanthus chrysotrichus* (Bignoniaceae)

Diana Salles Sampaio<sup>1</sup>, Maria Eugênia Costa<sup>2</sup> & Clesnan Mendes-Rodrigues<sup>1</sup>

<sup>1</sup> Universidade Federal de Uberlândia, Instituto de Biologia, Campus Umuarama, Bloco 2D, sala 28, Rua Ceará, s/n, CEP 38400-902, Uberlândia, Minas Gerais, Brazil.

<sup>2</sup> Universidade Estadual de Ponta Grossa, Departamento de Biologia Geral, Setor de Ciências Biológicas e da Saúde, Campus Uvaranas, Bloco M, Sala 95, Avenida General Carlos Cavalcanti, 4748, CEP 84030-900, Ponta Grossa, Paraná, Brazil.

Recebido em 26.XII.2012. Aceito em 18.XII.2013.

**ABSTRACT** – *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos is a polyembryonic species widely cultivated in urban areas, and with potential interest for reforestation purposes. The effects of light and temperature were tested on the germination rates and on the number of seedlings that emerge per seed. Germinability was higher in light (56%) and in temperatures between 25 and 30°C (59%). The highest mean numbers of seedlings per seed were observed from 20 to 30°C. Germinability and the mean seedling number per seed fit to the quadratic polynomial model, with higher germination rate estimated for 30.1°C (60.2%) and with the maximum number of seedlings per seed estimated at 26.3°C (1.9). These results point out that the seed germination rate was more affected by low temperatures than the mean seedling number per seed, but both were negatively affected by temperatures higher than 30°C.

Key words: polyembryony, seed germination, *Tabebuia*

**RESUMO** – Efeito da temperatura sobre o número de plântulas por semente em espécimes cultivados de *Handroanthus chrysotrichus* (Bignoniaceae). *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos é uma espécie poliembriônica amplamente cultivada em áreas urbanas e de potencial interesse para o reflorestamento. Nós testamos os efeitos da luz e da temperatura sobre as taxas de germinação e sobre o número de plântulas que emerge por semente. A germinabilidade foi maior na luz (56%) e em temperaturas entre 25 e 30°C (59%). Os maiores números médios de plântulas por semente foram observados entre 20 e 30°C. A germinabilidade e o número médio de plântulas por semente se ajustaram ao modelo quadrático polinomial, com a maior taxa de germinação estimada para 30,1°C (60,2%) e o maior número de plântulas por semente para 26,3°C (1,9). Os resultados sugerem que a taxa de germinação é mais afetada por baixas temperaturas que o número médio de plântulas por semente, mas ambos são negativamente afetados por temperaturas superiores a 30°C.

Palavras chave: germinação de sementes, poliembrião, *Tabebuia*

## INTRODUCTION

*Handroanthus chrysotrichus* (Mart. ex DC.) Mattos is a yellow trumpet tree widely cultivated in urban areas of Brazil (Gentry, 1992; Ruschel & Leite, 2002; Silva *et al.*, 2007). It occurs naturally in the Atlantic Rainforest and “Restinga”, especially on disturbed sandy soil areas (Gentry, 1992), considered a potential species for reforestation (Zamith & Scarano, 2006). The knowledge on the reproductive biology, germination and establishment of this species is essential for reforestation and ornamental purposes.

*Handroanthus chrysotrichus* is a self-compatible species with sporophytic apomixis (Bittencourt Jr. & Semir, 2005; Bittencourt Jr. & Moraes, 2010), which results in high polyembryonic seed percentages (Piazzano, 1998; Mendes-Rodrigues *et al.*, 2012). Polyembryony is reported as a parameter that can increase the percentage of seedling mortality due to embryo morphological anomalies or competition between embryos and seedlings (Bittencourt Jr. & Moraes, 2010; Mendes-Rodrigues *et al.*, 2012), but can also enhance the probability of at least one seedling to survive per seed in this species (Mendes-

Rodrigues *et al.*, 2012), described as the bet hedging effect for the polyembryonic *Vincetoxicum rossicum* (Kleopow) Barbar. (Ladd & Cappuccino, 2005).

Although investigations on the effect of light and germination temperature are available for *H. chrysotrichus* and related species (Santos *et al.*, 2005; Socolowski & Takaki, 2007; Pacheco *et al.*, 2008), there is no data about the effect of these variables on the number of seedlings that can emerge per seed. In this sense, the aims of the present study were to analyze the effects of light and temperature on germination rate and on the number of seedlings that emerge per seed in *H. chrysotrichus*.

## MATERIAL AND METHODS

Seeds were collected from ten cultivated individuals (at least two fruits per individual) on the Campus of the Pontificia Universidade Católica de Minas Gerais (19°55'16.75" S, 43°59'38.59" W), Belo Horizonte, Minas Gerais, Brazil, in November 1999. A voucher specimen was deposited in the Herbarium of the Department of Botany, Universidade Federal de Minas Gerais - BHCb, Belo Horizonte, MG, Brazil, under the accession number BHCb 68092. Seeds were pooled together and stored in paper bags in temperatures around 10°C for a maximum of three months before the germination experiment. Very small seeds or seeds without embryo were excluded.

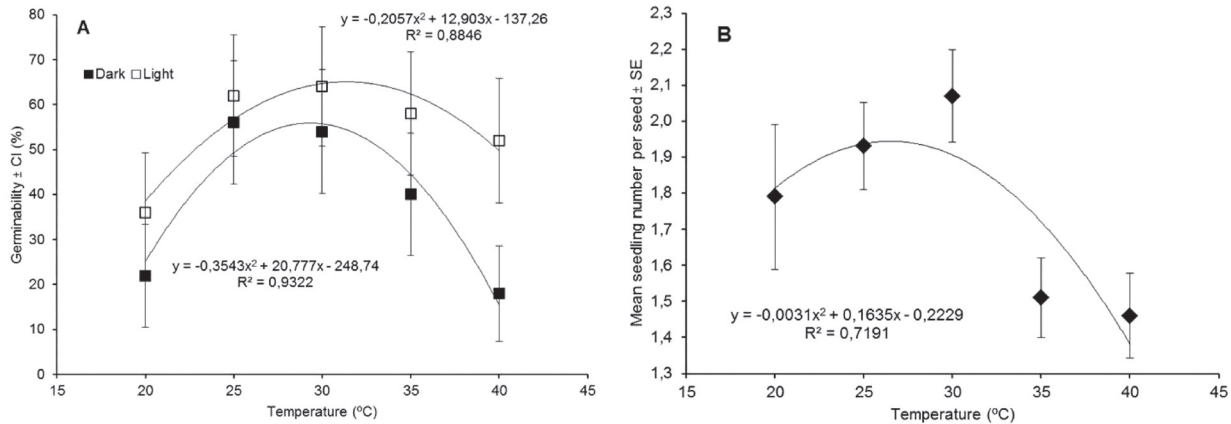
In order to evaluate the effect of light and temperature on germination rate and on mean seedling number per seed, seeds were submitted to 12 treatments including two factors. The first factor was temperature, using 20, 25, 30, 35, and 40 °C, in germination chambers (347CDG, FANEM), and room temperature (varying between 25 and 28°C). And the second factor was light condition, with continuous white light or darkness as the two light treatments. Two replicates with 25 seeds per treatment were used. Nevertheless, for room temperature under both lighting conditions, an extra replicate with 50 seeds was used, and for 35°C under both lighting conditions only one replicate of 50 seeds was used. Seed samples were sown inside plastic germination boxes, on filter paper moistened with 10 mL nystatin suspension (diluted 1 pill per litre of distilled water). The darkness treatment boxes were double-bagged individually in paper and two black plastic bags, and handled under green light, when necessary. All boxes were moistened as necessary during the experimental period with the same solution described above. Seeds

were considered germinated when at least one radicle protruded. Seedlings arising from each germinated seed were quantified when seed germination ceased in each treatment, between 11 and 21 days after sowing depending on the replicate.

A two-way ANOVA was performed to test the effects of light and temperature on germinability and mean seedling number per seed using the statistical package SISVAR 5.0 (Ferreira, 2008). The means were compared with the Scott-Knott test using the same program. For germinability, the temperature of 35°C was not included due to lack of replicates. Germinability and the mean seedling number per seed in different temperatures were also fitted to a quadratic polynomial model (excluding the room temperature treatment and separately for dark and light) and the significance of the fitting was tested with ANOVA using the statistical package BioEstat 5.0 (Ayres *et al.*, 2007). A second analysis was performed to estimate the temperature on which the highest germinability can occur and the greatest number of embryos can be converted into seedlings based in the quadratic polynomial models. The polyembryonic seed percentages were compared with Students' *t*-test for proportions (Snedecor & Cochran, 1989).

## RESULTS

The germinability was higher in light (56.1 ± 13.4%) than in darkness treatments (42.2 ± 18.8 %) ( $F_{1,12} = 7.92$ ,  $P < 0.016$ ), and differed among temperatures ( $F_{4,12} = 7.33$ ,  $P = 0.003$ ), with no interaction between light and temperature conditions ( $F_{4,12} = 1.06$ ,  $P < 0.419$ ). We observed lower germinability in temperatures of 20°C (29 ± 13.6%) and 40°C (35 ± 20.7%), while the other temperatures did not differ from each other (59% for all; with SD ± 8.7% for 25°C, ± 7.6% for 30°C, and ± 13.1% for room temperature) following the Scott-Knott test. The temperature of 35°C, which was not included in this analysis, showed 49% (± 12.7%) of germinability. The fit to the quadratic polynomial model showed significance to darkness ( $F_{2,2} = 41.22$ ,  $P = 0.021$ ) and light ( $F_{2,2} = 23.00$ ,  $P = 0.040$ ) and to both treatments together ( $y = -0.28x^2 + 16.84x - 193$ ,  $R^2 = 0.567$ ,  $F_{2,7} = 10.49$ ,  $P = 0.008$ ). The higher germination rates obtained from models were 55.9% to darkness in 29.2°C and 65.7% to light in 31.4°C (Fig. 1A). Independent of light treatment the higher germination rate estimated was 60.2% in 30.1°C.



**Fig. 1.** Quadratic polynomial model for (A) Germinability (± confidence interval) and (B) Mean seedling number per seed (± standard error) of *Handroanthus chrysotrichus* tested under five controlled temperatures.

The maximum number of seedlings found per seed for 20, 25°C, and room temperature was five, and for other temperatures were four. The mean seedling number developed per seed varied with temperature but not with lighting, without interaction between factors (Temperature:  $F_{5,329} = 3.80, P = 0.002$ ; Lighting:  $F_{1,329} = 0.85, P = 0.358$ ; Temperature x Lighting:  $F_{5,329} = 1.18, P = 0.319$ ). The lowest mean number of seedlings was observed at 35 and 40°C following the Scott-Knott

test (Tab. 1). The maximum number of seedlings per seed estimated from the quadratic polynomial model occurred at 26.3°C with 1.9 seedlings per seed ( $F_{1,221} = 4.38, P = 0.037$ ) (Fig. 1B). The polyembryonic seed percentage also did not vary with lighting ( $t = 0.34, df = 171.79, P = 0.733$ ), but varied with temperature (Tab. 1). The lowest polyembryonic seed percentage was observed at 35 and 40°C, and statistical analyses showed a marked decrease from 30 to 35°C (Tab. 1).

**Table 1.** Effect of temperature and lighting treatments on mean seedling number per seed and polyembryonic seed percentage of *Handroanthus chrysotrichus*.

| Effect      | Treatments                   | Mean seedling number per seed ± SD (n) <sup>a</sup> | Polyembryonic seed percentage (%) <sup>b</sup> |
|-------------|------------------------------|---|--|
| Temperature | 20°C                         | 1.8 ± 1.1 (29) A                                    | 44.8 ABC                                       |
|             | 25°C                         | 1.9 ± 0.9 (59) A                                    | 66.1 AB  |
|             | 30°C                         | 2.1 ± 1.0 (59) A                                    | 67.8 A   |
|             | 35°C                         | 1.5 ± 0.8 (49) B                                    | 36.7 C   |
|             | 40°C                         | 1.5 ± 0.7 (35) B                                    | 37.1 BC  |
|             | Room temperature (25 - 28°C) | 1.9 ± 0.9 (110) A                                   | 64.5 AB  |
| Lighting    | White light                  | 1.8 ± 0.8 (195) a                                   | 57.9 a   |
|             | Dark                         | 1.9 ± 1.0 (146) a                                   | 55.5 a   |

<sup>a</sup> Means followed by the same letter are not different based on Scott-Knott test ( $P < 0.05$ ). <sup>b</sup> Means followed by the same letter are not different based on t Student test for proportions ( $P < 0.05$ ). Capital letters refer to temperature comparisons and small letters to lighting comparisons.

**DISCUSSION**

Although a higher germinability was observed in light than in dark conditions in our analysis, Santos *et al.* (2005) did not verify any light effect on the germination rate of *H. chrysotrichus*. Germination experiments with species of the genera *Handroanthus* and *Tabebuia* have shown germination capacity

between 10 and 40°C (Santos *et al.*, 2005; Socolowski & Takaki, 2007; Pacheco *et al.*, 2008). But Santos *et al.* (2005) showed that the germination range is narrower for *H. chrysotrichus* (from 15 to 35°C), with maximum germination rates between 15 and 30°C and absence of germination at 10 and 40°C. This last study shows similar germinability rates (from 27.1 to 54.6%) to those found for the seed pool analysed

in the present work. Our data showed an increase of germination range for *H. chrysotrichus*, up to 40°C, but restricted the higher germination rates to 25 and 30°C. The fitting of seed germination rates in function of temperature to quadratic polynomial models is common for angiosperms (Baskin & Baskin, 1998) and Bignoniaceae species (Socolowski *et al.*, 2008) and can be justified by the injuries caused in cell membrane in high and low temperatures (Hendricks & Taylorson, 1976).

In the present experiment, higher values for the embryo to seedling conversion were found between 20 - 30°C. The quadratic polynomial models suggested 30.1°C for higher seed germination rate and 26.3°C for higher mean seedling number per seed, although the values were very close. These results point out that seed germination rate was more affected by low temperatures than the mean seedling number per seed, but both seemed to be negatively affected by temperatures higher than 30°C. Some experimental conditions used *in vitro* cultivation of embryos of *Ophiopogon japonicus* (L.f.) Ker-Gawl. and *Uapaca kirkiana* Müll. Arg. are also capable to change embryo to seedling turnover in polyembryonic seeds (Fukai *et al.*, 2000; Maliro & Kwapata, 2000). The fact that experimental conditions affected embryo to seedling turnover in *H. chrysotrichus*, suggests that environmental conditions can also affect the frequency of gemellar seedlings. As higher number of seedlings per seed enhances the probability of at least one seedling surviving per seed in this species (Mendes-Rodrigues *et al.*, 2012), higher temperatures can nullify the bet hedging advantages of polyembryony.

## CONCLUSION

This kind of study allows adjusting experimental conditions in order to obtain the highest seedling number per seed for each species. Moreover, as higher temperatures reduce the embryo to seedling turnover, relationships among temperature and seedling establishment in polyembryonic species can be useful to understand how seedling population's structure can be affected by changes in environment temperature.

## ACKNOWLEDGMENTS

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior with a post-doctoral fellowship (CAPES/PNPD n°

23038.008068/2010-95) to the first author (DSS) and a doctoral fellowship to the second author (MEC). We thank Dra. Cristina Generosa de Senna Queiroz for valuable discussions and for the use of the facilities of the Plant Physiology Lab. at Universidade Federal de Minas Gerais; Dr. Paulo Eugênio A. M. Oliveira (Universidade Federal de Uberlândia) for critical reading of the manuscript; Dr. H.D. Laughinghouse IV and R.F. Fischer for revising the English text; and the anonymous referees who provided important suggestions.

## REFERENCES

- Ayres, M., Ayres Júnior, M., Ayres, D.L. & Santos, A.A.S. 2007. BioEstat: Aplicações estatísticas nas áreas das ciências bio-médicas. Ong Mamirauá, Belém. 324p.
- Baskin, C.C. & Baskin, J.M. 1998. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego. 666p.
- Bittencourt Jr., N.S. & Semir, J. 2005. Late-acting, self-incompatibility and other breeding systems in *Tabebuia* (Bignoniaceae). International Journal of Plant Sciences, 166: 493-506.
- Bittencourt Jr., N.S. & Moraes, C.I.G. 2010. Self-fertility and polyembryony in South American yellow trumpet trees (*Handroanthus chrysotrichus* and *H. ochraceus*, Bignoniaceae): a histological study of post-pollination events. Plant Systematics and Evolution, 288: 59-76.
- Ferreira, D.F. 2008. SISVAR: um programa para análises e ensino de estatística. Revista Científica Symposium, 6: 36-41.
- Fukai, S., Shimomura, T. & Kondo, T. 2000. Morphological observation of polyembryony in *Ophiopogon japonicus* (Liliaceae). Journal of the Japanese Society for Horticultural Science, 69: 614-616.
- Gentry, A.H. 1992. Bignoniaceae – Part II (Tribe Tecomeae). Flora Neotropica. Monograph, 25: 1-370.
- Hendricks, S.B. & Taylorson, N.B. 1976. Variation in germination and amino acid leakage of seeds with temperature related to membrane phase change. Plant Physiology, 58: 7-11.
- Ladd, D. & Cappuccino, N. 2005. A field study of seed dispersal and seedling performance in the invasive exotic vine *Vincetoxicum rossicum*. Canadian Journal of Botany, 83: 1181-1188.
- Maliro, M.F.A. & Kwapata, M.B. 2000. Apomictic embryo development and survival in *Uapaca kirkiana* under *in vitro* and *in vivo* seed germination. Scientia Horticulturae, 83: 139-147.
- Mendes-Rodrigues, C., Sampaio, D.S., Costa, M.E., Caetano, A.P., Ranal, M.A., Bittencourt, Jr., N.S. & Oliveira, P.E. 2012. Polyembryony increases embryo and seedling mortality but also enhances seed individual survival in *Handroanthus* species (Bignoniaceae). Flora, 207: 264-274.

- Piazzano, M. 1998. Números cromosômicos em Bignoniaceae de Argentina. *Kurtziana*, 26: 179-189.
- Pacheco, M.V., Matos, V.P., Feliciano, A.L.P. & Ferreira, R.L.C. 2008. Germinação de sementes e crescimento inicial de plântulas de *Tabebuia aurea* (Silva Manso) Benth. & Hook f. ex S. Moore. *Ciência Florestal*, 18: 143-150.
- Ruschel, D. & Leite, S.L.C. 2002. Arborização urbana em uma área da cidade de Lajeado, Rio Grande do Sul, Brasil. *Caderno de Pesquisa, Série Biologia*, 14: 07-24.
- Santos, D.L., Sugahara, V.Y. & Takaki, M. 2005. Efeitos da luz e da temperatura na germinação de sementes de *Tabebuia serratifolia* (Vahl) Nich, *Tabebuia chrysotricha* (Mart. ex DC.) Standl. e *Tabebuia roseo-alba* (Ridl) Sand – Bignoniaceae. *Ciência Florestal*, 15: 87-92.
- Silva, L.M., Moccellini, R., Weissheimer, D.I., Zboralski, A.R., Fonseca, L. & Rodighiero, D.A. 2007. Inventário e sugestões para arborização em via pública de Pato Branco/PR. *Revista da Sociedade Brasileira de Arborização Urbana*, 2: 100-108.
- Socolowski, F. & Takaki, M. 2007. Germinação de sementes e emergência de plântulas de *Tabebuia rosea* (Bertoloni) A.P. De Candolle (Bignoniaceae), uma espécie exótica com potencial invasor. *Revista Árvore*, 31: 229-238.
- Socolowski, F., Vieira, D.C.M. & Takaki, M. 2008. Interaction of temperature and light on seed germination in *Tecoma stans* L. Juss. ex Kunth (Bignoniaceae). *Brazilian Archives of Biology and Technology*, 51: 723-730.
- Snedecor, G.W. & Cochran, W.G. 1989. *Statistical Methods*. Iowa State University, Ames. 503p.
- Zamith, L.R. & Scarano, F.R. 2006. Restoration of a Restinga sandy coastal plain in Brazil: survival and growth of planted woody species. *Restoration Ecology*, 14: 87-94.

