GERMINATION AND POST-SEMINAL DEVELOPMENT OF *Melaleuca alternifolia* (MAIDEN & BETCHE) CHEEL

Daniel Teixeira Pinheiro[2*], André Dantas de Medeiros[2], Paulo César Hilst[2], Antônio Lelis Pinheiro[3], and Denise Cunha Fernandes dos Santos Dias[2]

[1] Received on 16.08.2019 accepted for publication on 05.03.2020.
[2] Universidade Federal de Viçosa, Departamento de Agronomia, Viçosa, MG-Brasil. E-mail: <pinheiroagroufv@gmail.com> , <pchilst@yahoo.com.br> , <dcunhadias@gmail.com> and <medeiros.seeds@gmail.com>.
[3] Universidade Federal de Viçosa, Departamento de Engenharia Florestal, Viçosa, MG-Brasil E-mail: <pinheiroufv@gmail.com>.
*Corresponding author.

ABSTRACT – There is little information regarding the germination pattern, seed characterization, and seedling development of ***Melaleuca alternifolia***. This study aimed to determine the ideal temperature for the germination of ***M. alternifolia*** seeds, as well as to characterize the seeds and the post-seminal development of the species. Three lots of ***M. alternifolia*** seeds were placed to germinate at 20, 25, 20-30, 20-35, 30, and 35 °C, with daily evaluations to determine germination rate, germination speed index (GSI), speed of germination (SG), and mean time for germination of 50% (t50) and 100% (t100) of seeds. The inner morphology of the seeds was evaluated through X-ray images and seedling morphology by photographs. Alternating temperatures (20-30 and 20-35 °C) led to germination rates higher than the other temperatures. The GSI had the lowest values at the constant temperature of 20 °C and the highest values at the constant 30 °C for all the lots. The SG was lowest at a temperature of 20 °C and the highest at the temperature of 35 °C. At alternating temperatures (20-30 and 20-35 °C), t50 was around 5 days and t100 reached 16 days. In conclusion, the alternating temperatures of 20-30 °C and 20-35 °C are recommended for germination of ***M. alternifolia***, and final evaluation can be performed at 16 days after sowing. The inner structures of seeds could be visualized by X-ray analysis, and full seeds could be distinguished from empty seeds and impurities. Seeds exhibit epigeal germination and seedling development is stabilized after 30 days.

Keywords: Seedlings; X-rays; Seeds.

GERMINAÇÃO E DESENVOLVIMENTO PÓS-SEMINAL DE Melaleuca alternifolia *(MAIDEN & BETCHE) CHEEL.*

RESUMO – Informações sobre o padrão da germinação e caracterização de sementes e desenvolvimento de plântulas de ***Melaleuca alternifolia*** *são escassos. Objetivou-se com este trabalho determinar a temperatura ideal para a germinação de sementes* ***M. alternifolia****, além de caracterizar sementes e o desenvolvimento pós-seminal da espécie. Foram utilizados três lotes de sementes de* ***M. alternifolia*** *colocadas para germinar a 20, 25, 20-30, 20-35, 30 e 35 °C, com as avaliações diárias para determinação da taxa de germinação, índice de velocidade de germinação (IVG), velocidade de germinação (VG), tempo médio para germinação de 50 (t50) e 100% (t100). A morfologia interna das sementes foi avaliada por meio de imagens de raios X e a das plântulas por meio de fotografias. As temperaturas alternadas (20-30 e 20-35 °C) proporcionaram taxas de germinação superiores às demais. O IVG apresentou os menores e maiores valores nas temperaturas constantes de 20 e 30 °C para todos os lotes, respectivamente. O VG foi menor na temperatura de 20 °C e maior na temperatura de 35 °C. Nas temperaturas alternadas (20-30 e 20-35 °C), t50 foi em torno de 5 e t100 atingiu 16 dias. Como conclusão, as temperaturas alternadas de 20-30 e 20-35 °C são indicadas para a germinação de* ***M. alternifolia****, com a avaliação final podendo ser realizada aos 16 dias após a semeadura. A análise de raios-X permitiu visualizar as estruturas internas das sementes e distinguir sementes cheias e sementes vazias e impurezas. A germinação é epígea e as plântulas apresentam estabilização do desenvolvimento após 30 dias.*

Palavras-Chave: Plântulas; Raios X; Sementes.

1.INTRODUCTION

The *Melaleuca* genus belongs to the Myrtaceae family and is made up of around 230 species (Amri et al., 2012). Among them, *Melaleuca alternifolia* (Maiden & Betche) Cheel, native to Australia, is considered one of the most important species of the genus, due to the extensive length of time it has been used at its place of origin (Sharifi-Rad et al., 2017). It is an arboreal species that can reach a mean height of six meters and is mainly used for extraction of essential oil from its leaves, popularly known as “tea tree oil” (Chen et al., 2016). Recent studies cite diverse traits of and uses for “tea tree oil”, such as antifungal and antibacterial activity (Li et al., 2016), insect repellence (Yim et al., 2016), healing properties (Han and Parker, 2017), and others.

Germination is considered a fundamental step in crop establishment within the context of a whole plant cycle. During this process, a mature seed can be highly sensitive to environmental factors, which will affect mobilization of reserves, gene regulation, protein synthesis, and other important components of the process (Bewley et al., 2013; Finch-Savage and Bassel, 2016). Temperature is considered one of the main factors in germination, since it affects both germination percentage and speed. The thermal limits for germination are defined by the cardinal temperatures (optimum, maximum, and minimum), which determine the ecological limitations for geographic distribution and establishment of the species (Dürr et al., 2015; Marcos-Filho, 2016; Daibes and Cardoso, 2018). In general, temperatures outside the range considered optimal for a species promote or inhibit important biochemical processes, such as glycolysis and the tricarboxylic acid cycle, as well as the activity of various enzymes and other processes that are directly related to the germination capacity of dormant and non-dormant seeds (Bewley et al., 2013; Xia et al., 2018).

Since *M. alternifolia* seeds are extremely small and difficult to handle, information regarding the pattern of their germination and the effects of temperature on this process is very limited in the literature. *M. alternifolia* germination has been evaluated without, however, evaluating the effect of different temperatures (Anselmini et al., 2010). The effect of temperature and of light on the species *Melaleuca quinquenervia* has also been evaluated, in which the temperature of 27.3 °C allowed maximum germination in the shortest period of time, and it is considered ideal for that species (Martins et al., 2013).

Another important factor for seedling production and plant establishment under natural conditions is knowledge regarding the morphological aspects of seeds and seedlings (Abud et al., 2010). In regard to seeds, the use of non-destructive analyses, such as the X-ray test, has been documented as effective for studies of internal morphology of seeds of diverse species, such as *Moquiniastrum polymorphum* (Faria et al., 2019), *Moringa oleifera* (Noronha et al., 2018), and *Leucaena leucocephala* (Medeiros et al., 2018), among others. This technique assists in identification of full seeds, empty seeds, mechanical damage, and spots that indicate deterioration, characteristics that can affect seed physiological potential (Marchi and Gomes Junior, 2017), and, consequently, the quality of seed lots.

Information regarding morphology of seedlings in the initial stages of development are also relevant for interpretation and standardization for correct evaluation of seed physiological quality (Abud et al., 2010), and they also contribute to identification of the species under natural conditions.

In light of the above and since information for this species is quite limited, the aim of this study was to evaluate the pattern of germination and define adequate temperature for germination of *M. alternifolia* seeds, as well as characterize the seeds and the post-seminal development of the species.

2.MATERIALS AND METHODS

The study was conducted in 2017 in the Seed Analysis Laboratory of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. Three lots of *M. alternifolia* seeds were used, collected in the municipality of Coimbra, Minas Gerais, Brazil (20º51'24"S, 42º48'10"W, 720 m). Climate in the region is classified as hot and temperate, with mean temperature of 20.1°C and mean rainfall of 1283 mm. The seed lots corresponded to three groups located in different areas of the municipality and were constituted by seeds from at least five different mother plants each. After harvest, the seeds were dried in the shade and processed using sieves for removal of larger impurities. After that, they were stored at 20 °C for approximately three months, up to the time of evaluations.

For evaluation of the effect of temperature on germination, the seeds of the three lots were distributed on two sheets of paper towel in 15-cm-diameter Petri dishes. The paper was moistened with distilled water at the volume of 2.5 times the weight of the dry paper.

Due to the reduced size of the seeds (around 0.5 mm) and the difficulty of conducting individual evaluations, the germination test was performed with samples based on weight, as described in the Rules for Seed Testing (Regras para Análise de Sementes) for the test with weighed replications (Brasil, 2009). For that purpose, pre-tests were conducted and the weight of 0.0250 g of seed to be used for each replication was defined. The seeds were kept in BOD with an eight-hour photoperiod of white light at the temperatures of 20, 25, 20-30, 20-35, 30, and 35 °C. At alternating temperatures (20-30 and 20-35 °C), the lighting period and the highest temperature coincided (Brasil, 2009).

Due to the difficulty of defining a standard for normal seedlings for evaluation of the test, the number of germinated seeds (root protrusion) was considered through daily counts, up to stabilization of the counts of all the treatments. The daily germination evaluations were performed with the aid of a tabletop magnifying glass with 8x magnification, and the results were expressed in germinated seeds per 0.0250 g of seeds.

The data of daily germination counts carried out for the best temperatures were evaluated to obtain the following variables: germination speed index (GSI) (Maguire, 1962), speed of germination (SG) (Edmond and Drapala, 1958), t50 (time for 50% germination of the lot) and t100 (time for 100% germination of the lot).

For analyses of inner seed morphology, radiographic images were generated using the Faxitron MX-20 device (Faxitron X-ray Corp. Wheeling, IL, U.S.A). Seeds were placed under radiation for 10 seconds at 32 kV and a focal distance of 14 cm. The digital images generated were saved on a computer and used for descriptive identification of the physical components (full seeds, empty seeds, and impurities) contained in the sample.

Evaluation of post-seminal development was conducted together with germination. Seeds and seedlings in different stages were selected and photographed under high resolution. The main structures of the seeds and seedlings were identified through the photographs.

The experiment was conducted in a completely randomized design (CRD) in a 6 × 3 factorial arrangement, consisting of 6 temperatures (20, 25, 20-30, 20-35, 30, and 35 °C) and 3 lots, with 10 replications. Each experimental unit was composed of a Petri dish containing 0.0250 g of seed. Analysis of variance was performed on the data. The mean values were compared by the Tukey test at 5% probability with assistance of the R 3.5.1 software.

3.RESULTS

Analysis of germination of *M. alternifolia* over time showed that, for all the lots, alternating temperatures (20-30 and 20-35 °C) led to germination rates higher than the rates from the other treatments, especially from the fifth day after sowing on. In contrast, the constant temperatures of 20 °C and 35 °C generally led to lower germination rates. The temperatures of 25 °C and 30 °C generally exhibited intermediate germination rates compared to the other treatments (Figure 1).

The temperature of 20 °C resulted in slower germination in all the seed lots, with germination mainly increasing as of the eighth day after sowing. At the other temperatures, this accentuated increase occurred around the fourth day. In lot 1, the temperature of 20-30 °C tended to be superior to 20-35 ºC (Figure 1A). In lot 2, this response was the opposite (greater germination at the temperature of 20-35 ºC) (Figure 1B), and in lot 3, these treatments led to a similar response (Figure 1C).

Evaluation of the final germination of the three lots analyzed shows that the performance or response of each one of the lots was maintained at all the temperatures evaluated – lot 1 had germination greater than the others and lot 3 was lower than lots 1 and 2 (Figure 2).

Alternating temperatures (20-30 and 20-35 °C) led to higher germination rates and did not differ from each other. In the three seed lots analyzed, germination at the temperature of 35 °C was statistically lower than at the other temperatures analyzed (Figure 2A), contributing to reduction in *M. alternifolia* seed germination and indicating that 35 °C is above the temperature considered optimal for the species (Figure 2).

The germination speed index (GSI) had the lowest values at the constant temperature of 20 °C and the highest values at the constant temperature of 30°C for the three lots analyzed (Figure 3A). Speed of germination (SG), which represents the number of days necessary to germinate, was generally lower (greater number of days) at the temperature of 20 °C and greater (fewer number of days) at the temperature of 35 °C, respectively (Figure 3B).

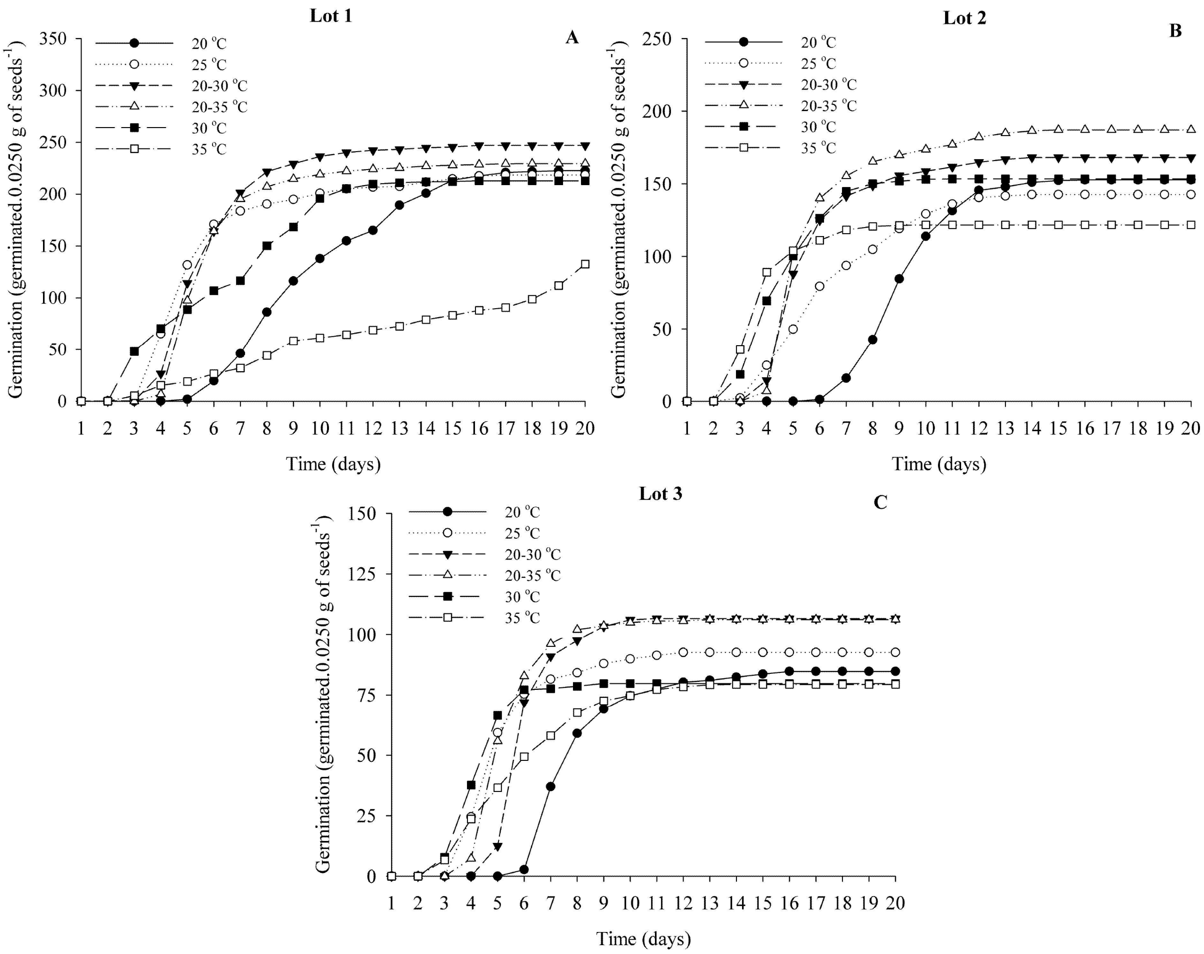

Figure 1 – Germination of seeds from three lots of *M. alternifolia* over time and at different temperatures.
Figura 1 – *Germinação de três lotes de* ***M. alternifolia*** *ao longo do tempo e em diferentes temperaturas.*

Under the temperature of 20-30 °C, the t50 (indicating 50% germination) was 5.24, 4.97, and 5.72 days for lots 1, 2, and 3, respectively (Figure 4A). In a similar manner, at 20-35 °C, these values were 5.26, 4.91, and 4.97 days (Figure 4B).

Evaluation of t100 (indicating 100% germination) shows values of 16.05, 13.94, and 10.01 days at the temperature of 20-30 °C (Figure 4C) and 13.99, 12.70, and 9.53 days at the temperature of 20-35 °C for lots 1, 2, and 3, respectively (Figure 4D).

From analysis of internal morphology of *M. alternifolia* seeds by the X-ray test, it can be seen that power adjusted to 32 kV, exposure time of 10 seconds, and focal distance of 14 cm allowed visualization of internal seed structures and differentiation of full seed and empty seed groups, as well as identification of impurities in the sample (Figure 5).

Seedlings of *M. alternifolia* in their initial stage of development up to 30 days after sowing (when development stabilized) under the alternating temperatures of 20-30 °C are shown in Figure 5D. Seed germination was found to be of the epigeal type, and the seed coat adheres to the cotyledons in the initial phase of seedling growth, where the hypocotyl-radicle axis emerges from the substrate. The two cotyledons emerge and expand. The normal seedling has a robust main root and few secondary roots. The hypocotyl is of a greenish-white color and cylindrical, and the partially expanded cotyledons expose the convex surface, exhibiting epigeal germination.

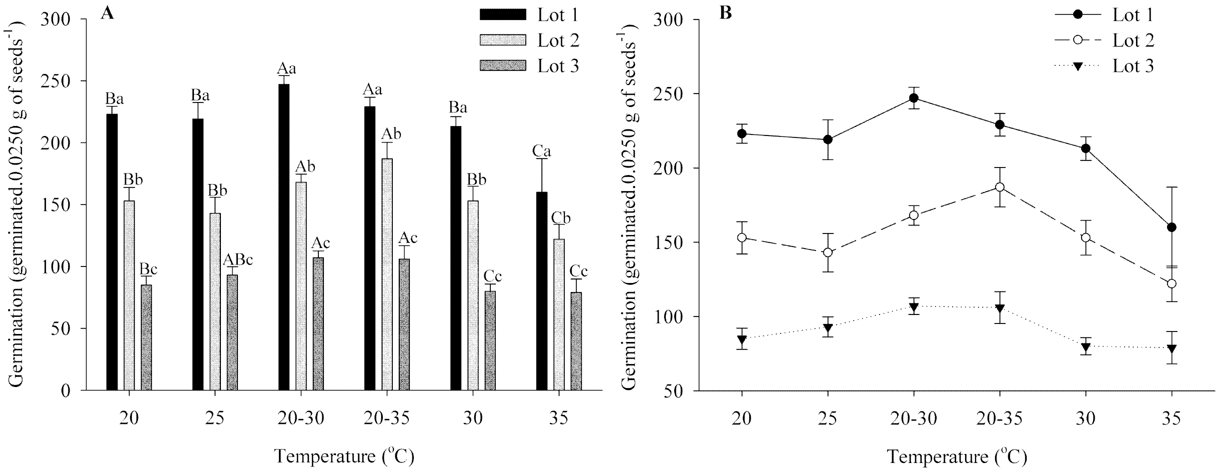

Figure 2 – Germination of seeds from three lots of *M. alternifolia* at different temperatures. Mean values followed by the same lowercase letter between lots and uppercase letters between temperatures do not differ from each other by the Tukey test at 5% probability (A). Trend curves of the three lots analyzed at the different temperatures (B). Bars: standard deviation.

Figura 2 – *Germinação de sementes de três lotes de* ***M. alternifolia*** *em diferentes temperaturas. Médias seguidas pela mesma letra minúscula entre lotes e maiúsculas entre temperaturas não diferem entre si pelo teste de Tukey a 5% de probabilidade (A). Curvas de tendência dos três lotes analisados nas diferentes temperaturas (B). Barras: desvio padrão.*

4.DISCUSSION

The lower germination rates observed at the temperatures of 20 °C and 35 °C indicate that these temperatures inhibited germination of *M. alternifolia*, probably for being outside the optimum range for this species. Similar results were observed in *Dalbergia odorifera* seeds, for which the germination rate declined both at 15 °C and at 35 °C (Liu et al., 2017). In contrast, the ideal temperature for germination of *Melaleuca ericifolia* was approximately 20 °C, showing the difference in response even between species of the same genus (Robinson et al., 2006).

In general, temperatures below those considered optimal reduce speed of germination and lead to

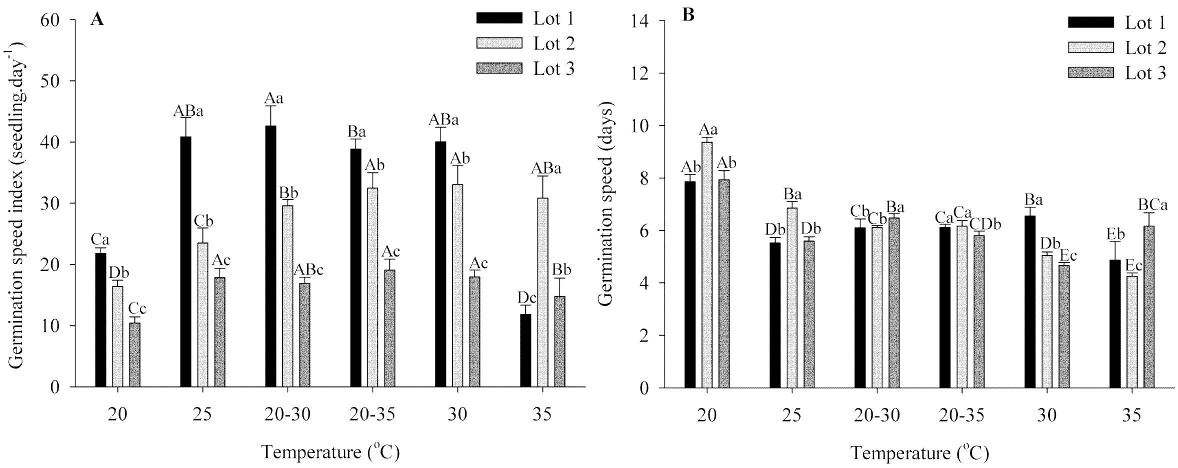

Figure 3 – Germination speed index (GSI) and speed of germination (SG) of seed from three lots of *M. alternifolia* at different temperatures. Mean values represented by the same lowercase letter between lots and uppercase letters between temperatures do not differ from each other by the Tukey test at 5% probability. Bars: standard deviation.

Figura 3 – *Índice de velocidade de germinação (IVG) e velocidade de germinação (VG) de três lotes de* ***M. alternifolia*** *em diferentes temperaturas. Médias representadas pela mesma letra minúscula entre lotes e maiúsculas entre temperaturas não diferem entre si pelo teste de Tukey a 5% de probabilidade. Barras: desvio padrão.*

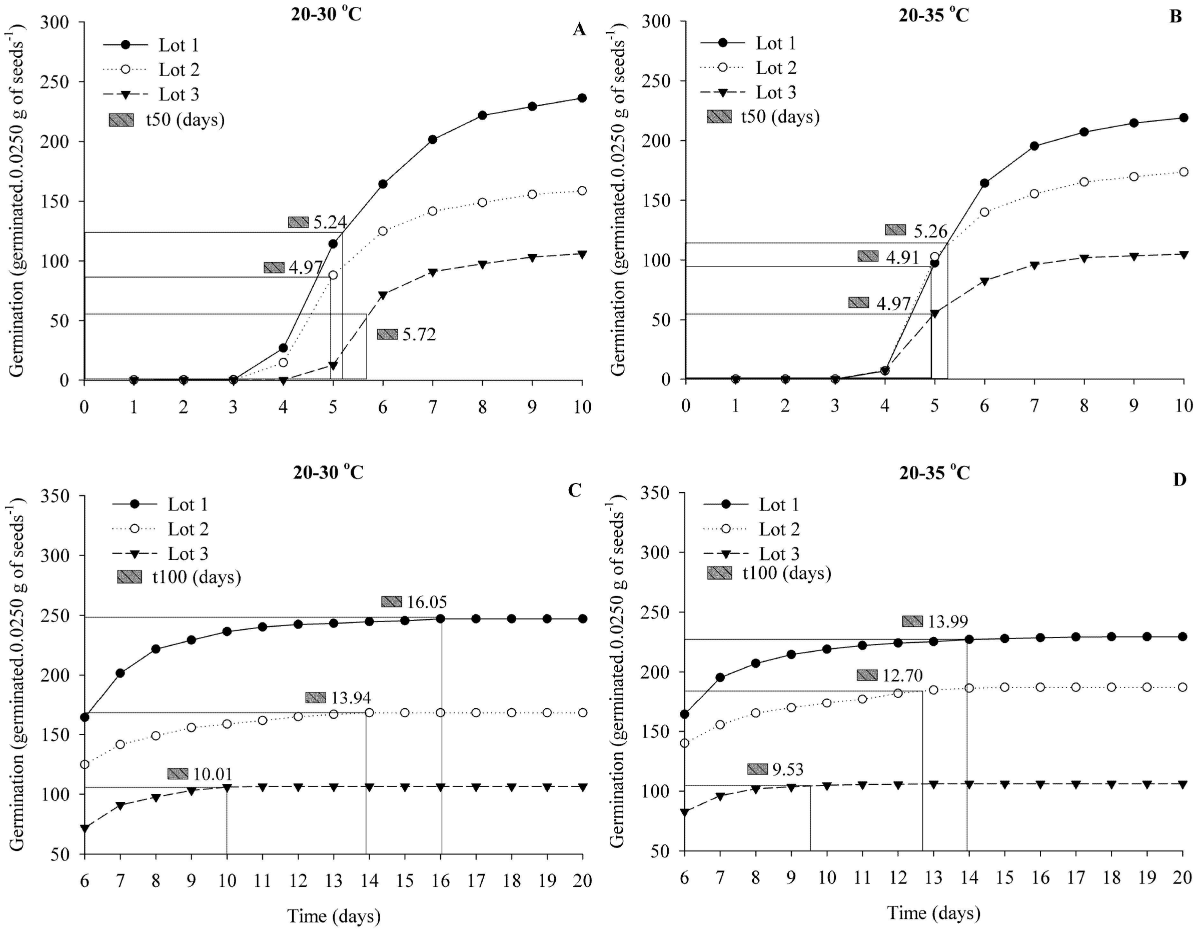

Figure 4 – Time necessary for 50% germination (t50) and 100% germination (t100) of three seed lots of *M. alternifolia* under 20-30 °C (A and C) and 20-35 °C (B and D).
Figura 4 – *Tempo necessário para germinação de 50% (t50) e 100% (t100) de três lotes de sementes de* ***M. alternifolia*** *sob 20-30 ºC (A e C) e 20-35 oC (B e D).*

physiological changes in the seeds, such as deactivation of gibberellin (GA), abscisic acid (ABA) synthesis, reduction in starch hydrolysis, and other processes that, in combination, contribute to reduction in germination (Wang et al., 2018). In germination of the species *Paeonia ostii*, it was observed that in addition to effects related to ABA induction and lower starch degradation, low temperature induced lower protein synthesis and transcription of genes involved in the germination process (Ren et al., 2018). In addition, lower speed of germination induced by low temperatures increases the possibility of infestation by fungi, due to greater exposure time of seeds to the environment they are in (Souza et al., 2016).

In a way similar to the results observed in this study, *Melaleuca quinquenervia* exhibited reduction in germination under 35 °C and 40 °C (Martins et al., 2013). In general, temperatures above the ideal lead to greater speed of germination, yet, at the same time, reduce the percentage of germination and vigor through diverse physiological, biochemical, and genetic processes (Bita and Gerats, 2013). Among their deleterious effects, high temperatures can suppress germination of dormant or non-dormant seeds through thermoinhibition, which, for its part, is related to expression of genes involved in ABA biosynthesis (Kendall and Penfield, 2012). In addition, high temperatures contribute to an increase in respiratory rates and, consequently, production of reactive species,

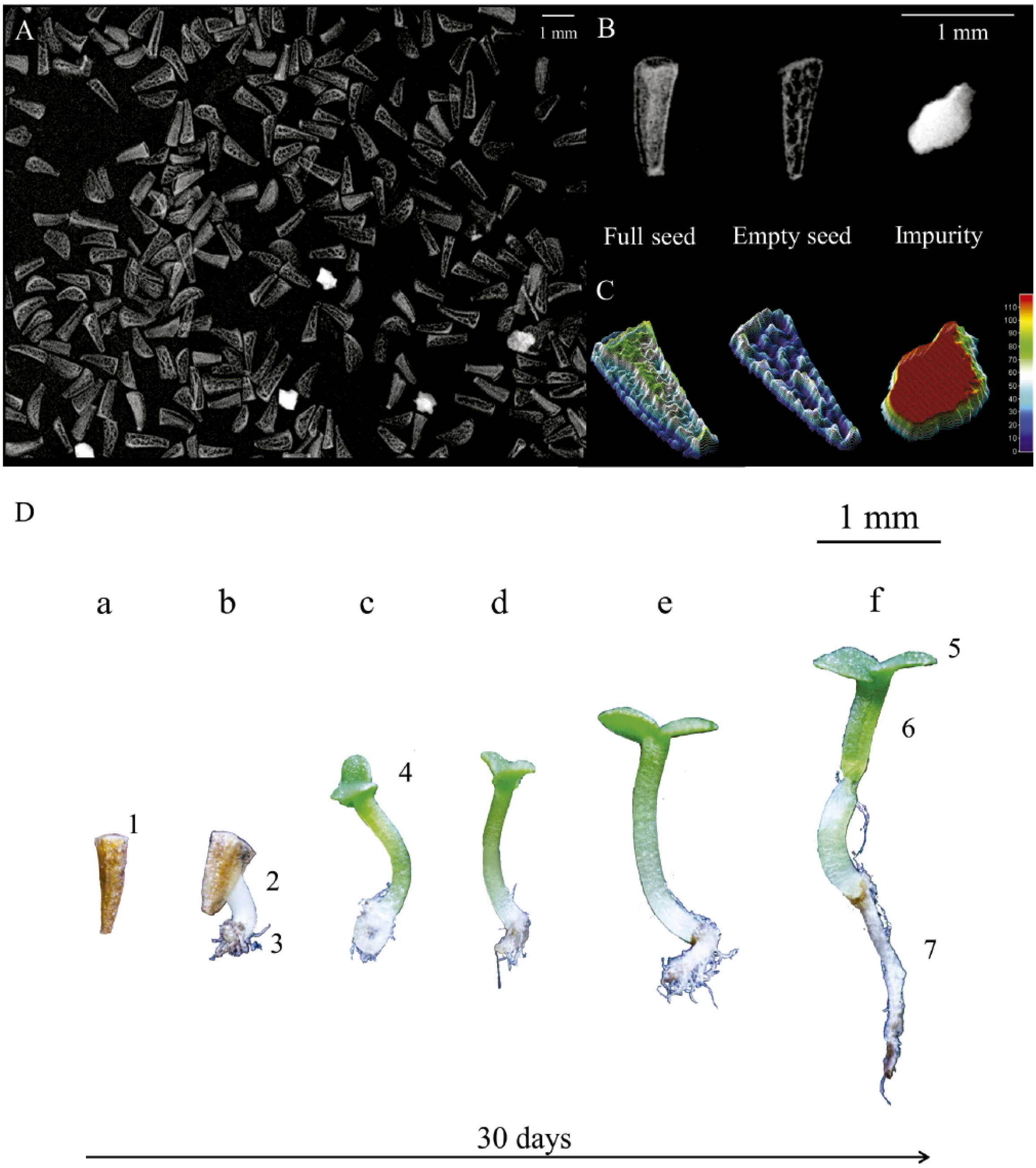

Figure 5 – Radiographic images of *M. alternifolia* seeds generated by the X-ray technique (A, B, and C) and characterization of post-seminal development of *M. alternifolia* seedlings (D). Wide view of the lot (A), classification of the seed lot components (B), 3D representation of the tissue density of the components (C). Seed (a), seed coat rupture and primary root emergence (b), seedling development and cotyledon expansion (c - f). Structures identified: seed coat (1), hypocotyl-radicle axis (2), radicle (3), cotyledon in expansion (4), totally expanded cotyledon (5), hypocotyl (6), and root system (7).

Figura 5 *– Imagens radiográficas de sementes de **M. alternifolia** geradas pela técnica de raios X (A, B e C) e caracterização do desenvolvimento pós-seminal de plântulas de **M. alternifolia**. (D). Visão ampla do lote (A), classificação dos componentes do lote de sementes (B), representação 3D da densidade tecidual dos componentes (C). Semente (a), rompimento do tegumento e protrusão de raiz primária (b), desenvolvimento das plântulas e expansão dos cotilédones (c - f). Estruturas identificadas: tegumento (1), eixo hipocótilo radícula (2), radícula (3), cotilédone em expansão (4), cotilédone totalmente expandido (5), hipocótilo (6), sistema radicular (7).*

which can cause irreversible physiological damage, such as denaturation of proteins and enzymatic inhibition (Mittler, 2017). High temperatures may also affect seedling development in relation to stomatal density, as observed in *Melaleuca lanceolata* plants (Hill et al., 2014).

It is important to emphasize that seeking to maximize seedling production, as well as a satisfactory germination rate, one of the main objectives is to obtain fast and uniform germination, which, for its part, will be optimized under optimal temperature conditions (Rajjou et al., 2012; Martins et al., 2013). In this context, in spite of leading to the best results for GSI and SG (Figure 3), the constant temperatures of 30 °C and 35 °C cannot be considered ideal for the germination of *M. alternifolia* since they led to lower rates of germination compared to the alternating temperatures (Figure 2). Unlike that observed here, the constant temperature of 27.3 °C was considered ideal for germination of *Melaleuca quinquinervia* (Martins et al., 2013). Nevertheless, these authors did not evaluate alternating temperatures and different seed lots.

Thus, alternating temperatures (20-30 and 20-35 °C) were selected as most adequate for germination of *M. alternifolia*, since in a general context, they combined greater percentage and greater speed of germination (Figures 2 and 3). Seeds of *Mauritia flexuosa* also germinated better under alternating temperatures, and their effects may be related to enzymatic mechanisms capable of being activated at different temperatures (Almeida et al., 2018).

In addition to confirming the similarity of results for the temperatures of 20-30 and 20-35 °C, there is an indication that final evaluation of *M. alternifolia* germination can be carried out at around the sixteenth day after sowing, since there were no increases in germination of the three seed lots analyzed from that time on, at either of the temperatures (Figure 4C and D).

Temperature is also considered an important environmental factor related to the different dormancy mechanisms. MacGregor et al. (2015) observed that in *Arabidopsis* seeds, low temperatures caused an increase in gene expression of enzymes and compounds related to breaking dormancy. In other species, such as *Carex diandra* (Fernández-Pascual et al., 2015) and *Anisantha rubens* (Jiménez-Alfaro et al., 2018), it has been shown that alternating temperatures increase germination through breaking dormancy, which, for its part, is mainly related to transcription of genes involved in metabolism, plant hormones, and the circadian clock (Foley et al., 2010). However, it is important to reinforce that the aim of the present study was not to evaluate dormancy and, thus, the results observed may serve as a tool for future evaluations.

The application of the X-ray test in evaluation of seed quality is interesting as a precise method, where seeds may be examined individually in expanded images that are able to indicate, in detail, the location and extent of damaged or altered structures (Cicero, 2010). Recent studies carried out with *Moquiniastrum polymorphum* (Faria et al., 2019), *Leucaena leucocephala* (Medeiros et al., 2018), and *Senna multijuga* (Marchi and Gomes Junior, 2017) also showed efficient application of the use of radiographic images for analysis of internal seed morphology. Although the option in this study was for more descriptive evaluation regarding aspects related to seed radiographic images, the study is relevant because it deals with introduction of the technique in the species in a pioneering way and can serve as a basis for future studies. The growth of *M. alternifolia* seedlings stabilized after 30 days under the condition of 20-30 °C, probably requiring transfer to substrate beginning at this time. This information is important mainly in regard to optimization of the seedling production process, aiming at higher rates and speed of germination.

Based on the results observed and due to the limited amount of information regarding seed characteristics and initial seedling development of *M. alternifolia*, this study may assist future research applied to seed technology of this species.

5.CONCLUSIONS

Alternating temperatures of 20-30 and 20-35 °C with 8-h photoperiod are recommended for germination of *M. alternifolia*, leading to higher rates and greater speeds of germination. Under these temperatures, final evaluation of germination can be carried out at 16 days after sowing. X-ray images allow identification of full seeds, empty seeds, and impurities contained in *M. alternifolia* seed lots and can serve as a support for development of automated analyses and assist in selection of lots with a higher level of purity. Germination of *M. alternifolia* seeds is epigeal, and seedlings exhibit stabilization of development after 30 days under alternating temperature of 20-30 °C.

6.REFERENCES

Abud HF, Gonçalves NR, Reis, RGE, Gallão MI, Innecco R. Morphology of seed and seedling of safflower. Revista Ciência Agronômica. 2010;41(2):259–65. doi: 10.1590/S1806-66902010000200013

Almeida LCP, Pivetta KFL, Gimenes R, Romani GN, Ferraz MV, Mazzini-Guedes RB. Temperature, light, and desiccation tolerance in seed germination of *Mauritia flexuosa* L.F. Revista Árvore. 2018;42(3):e420305. doi: 10.1590/1806-90882018000300005

Amri I, Mancini E, Martino L, Marandino A, Lamia H, Mohsen H. et al. Chemical Composition and Biological Activities of the Essential Oils from Three Melaleuca Species Grown in Tunisia. International Journal of Molecular Sciences. 2012;13(12):16580-591. doi: 10.3390/ijms131216580

Anselmini JI, Deschamps C, Gavazza MIA, Zanette F, Panobianco M. Dormência e germinação de sementes de Melaleuca alternifolia Cheel. Revista Brasileira de Plantas Medicinais. 2010;12(2):149–52.

Bewley JD, Bradford K, Hilhorst H, Nonogaki H. Seeds: physiology of development, germination and dormancy. New York: Springer; 2013.

Bita CE, Gerats T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science. 2013;4:273-1-18. doi: 10.3389/fpls.2013.00273

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasília, DF: MAPA/ACS; 2009.

Chen B, Li J, Zhang J, Fan H, Wu L, Li Q. Improvement of the tissue culture technique for *Melaleuca alternifolia*. Journal of Forestry Research. 2016;27(6):1265–69. doi: 10.1007/s11676-016-0301-7

Cicero SM. Aplicação de imagens radiográficas no controle de qualidade de sementes. Informativo ABRATES. 2010;20(3):48–51.

Daibes LF, Cardoso VJM. Seed germination of a South American forest tree described by linear thermal time models. Journal of Thermal Biology. 2018;76:156–64. doi: 10.1016/j.jtherbio.2018.07.019

Dürr C, Dickie JB, Yang XY, Pritchard HW. Ranges of critical temperature and water potential values for the germination of species worldwide: contribution to a seed trait database. Agricultural and Forest Meteorology. 2015;200:222–32. doi: 10.1016/j.agrformet.2014.09.024

Edmond JB, Drapala WJ. The effects of temperature, sand and soil, and acetone on germination of okra seed. Proceedings of the American Society Horticutural Science. 1958;71(1):428–34.

Faria JCT, Melo LA, Assumpção CRM, Brondani GE, Breier TB, Faria JMR. Physical quality of seeds of Moquiniastrum polymorphum. Brazilian Journal of Biology. 2019;79(1):63-69. doi: 10.1590/1519-6984.175407

Fernández-Pascual E, Seal CE, Pritchard, HW. Simulating the germination response to diurnally alternating temperatures under climate change scenarios: comparative studies on *Carex diandra* seeds. Annals of Botany. 2015;115(2):201–09. doi: 10.1093/aob/mcu234

Finch-Savage WE, Bassel GW. Seed vigour and crop establishment: Extending performance beyond adaptation. Journal of Experimental Botany. 2016;67(3):567–91. doi: 10.1093/jxb/erv490

Foley ME, Anderson JV, Chao WS, Doğramaci M, Horvath DP. Initial changes in the transcriptome of Euphorbia esula seeds induced to germinate with a combination of constant and diurnal alternating temperatures. Plant Molecular Biology. 2010;73(1–2):131–42. doi: 10.1007/s11103-009-9569-8

Han X, Parker TL. Melaleuca (*Melaleuca alternifolia*) essential oil demonstrates tissue-remodeling and metabolism-modulating activities in human skin cells. Cogent Biology. 2017;3(1):1318476. doi: 10.1080/23312025.2017.1318476

Hill KE, Hill RS, Watling JR. Do CO_2, temperature, rainfall and elevation influence stomatal traits and leaf width in Melaleuca lanceolata across southern Australia? Australian Journal of Botany. 2014;62(8):666-73. doi: 10.1071/BT14300

Jiménez-Alfaro B, Hernández-González M, Fernández-Pascual E, Toorop P, Frischie S, Gálvez-Ramírez C. Germination ecology of winter annual grasses in Mediterranean climates: Applications for soil cover in olive groves. Agriculture, Ecosystems & Environment. 2018;262:29–35. doi: 10.1016/j.agee.2018.04.013

Kendall S, Penfield S. Maternal and zygotic temperature signalling in the control of seed dormancy and germination. Seed Science Research. 2012;22(S1):S23–S29. doi: 10.1017/S0960258511000390

Li WR, Li HL, Shi QS, Sun TL, Xie XB, Song B, et al. The dynamics and mechanism of the antimicrobial activity of tea tree oil against bacteria and fungi. Applied Microbiology and Biotechnology. 2016;100(20):8865–75. doi: 10.1007/s00253-016-7692-4

Liu X, Xu D, Yang Z, Zhang N. Geographic variations in seed germination of *Dalbergia odorifera* T. Chen in response to temperature. Industrial Crops and Products. 2017;102:45–50. doi: 10.1016/j.indcrop.2017.03.027

MacGregor DR, Kendall SL, Florance H, Fedi F, Moore K, Paszkiewicz K, et al. Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. New Phytologist. 2015;205(2):642–52. doi: 10.1111/nph.13090

Maguire JD. Speed of germination-aid selection and evaluation for seedling emergence and vigor. Crop Science. 1962;2:176–77.

Marchi JL, Gomes Junior FG. Use of image analysis techniques to determine the embryo size of *Senna multijuga* (Rich.) seeds and its relation to germination and vigor. Journal of Seed Science. 2017;39(1):13–19. doi: 10.1590/2317-1545v39n1165423

Marcos-Filho J. Seed Physiology of Cultivated Plants. Londrina: Abrates; 2016. ISBN: 9788564895058

Martins CC, Martins D, Souza GSF, Costa NV. Eco-physiological aspects of melaleuca seed germination. Journal of Food, Agriculture & Environment. 2013;11(1):1157–61.

Medeiros AD, Araújo JO, Zavala-León MJ, Silva LJ, Dias DCFS. Parameters based on x-ray images to assess the physical and physiological quality of *Leucaena leucocephala* seeds. Ciência e Agrotecnologia. 2018;42(6):643–52. doi: 10.1590/1413-70542018426023318

Mittler R. ROS Are Good. Trends in Plant Science. 2017;22(1):11–19. doi: 10.1016/j.tplants.2016.08.002

Noronha BG, Medeiros AD, Pereira MD. Avaliação da qualidade fisiológica de sementes de *Moringa oleifera* Lam. Ciência Florestal. 2018;28(1):393-402.

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C, et al. Seed Germination and Vigor. Annual Review of Plant Biology. 2012;63(1):507–33. doi: 10.1146/annurev-arplant-042811-105550

Ren XX, Xue JQ, Wang SL, Xue YQ, Zhang P, Jiang HD, et al. Proteomic analysis of tree peony (*Paeonia ostii* 'Feng Dan') seed germination affected by low temperature. Journal of Plant Physiology. 2018;224–225:56–67. doi: 10.1016/j.jplph.2017.12.016

Robinson RW, Boon PI, Bailey P. Germination characteristics of *Melaleuca ericifolia* Sm. (swamp paperbark) and their implications for the rehabilitation of coastal wetlands. Marine and Freshwater Research. 2006;57(7):703-11. doi: 10.1071/MF06006

Sharifi-Rad J, Salehi B, Varoni EM, Sharopov F, Yousaf Z, Ayatollahi SA, et al. Plants of the Melaleuca Genus as Antimicrobial Agents: From Farm to Pharmacy. Phytotherapy Research. 2017;31(10):1475–94. doi: 10.1002/ptr.5880

Souza LF, Gasparetto BF, Lopes RR, Barros IBI. Temperature requirements for seed germination of *Pereskia aculeata* and *Pereskia grandifolia*. Journal of Thermal Biology. 2016;57:6–10. doi: 10.1016/j.jtherbio.2016.01.009

Wang Y, Cui Y, Hu G, Wang X, Chen H, Shi Q, et al. Reduced bioactive gibberellin content in rice seeds under low temperature leads to decreased sugar consumption and low seed germination rates. Plant Physiology and Biochemistry. 2018;133:1–10. doi: 10.1016/j.plaphy.2018.10.020

Xia Q, Ponnaiah M, Cueff G, Rajjou L, Prodhomme D, Gibon, Y, et al. Integrating proteomics and enzymatic profiling to decipher seed metabolism affected by temperature in seed dormancy and germination. Plant Science. 2018;269:118–25. doi: 10.1016/j.plantsci.2018.01.014

Yim WT, Bhandari B, Jackson L, James P. Repellent effects of *Melaleuca alternifolia* (tea tree) oil against cattle tick larvae (*Rhipicephalus australis*) when formulated as emulsions and in β-cyclodextrin inclusion complexes. Veterinary Parasitology. 2016;225:99–103. doi: 10.1016/j.vetpar.2016.06.007