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Germination and emergence of Miconia sp (Melastomataceae)

Melastomataceae is one of the predominant families of Campos Gerais Paranaenses. However, germination and initial growth of the species in the region have been little studied. To investigate the germination, emergence, and initial growth of five Melastomataceae species in the State Park of Guartelá and in the State Park of Vila Velha, germination bioassays and emergence tests were carried out at 20 °C, 25 °C, and 30 °C in a germination chamber and initial growth wasevaluated in a greenhouse. Miconia cinerascens and Miconia sellowiana had the highest percentage of germination (% G) at 25 °C and 30 °C. M. auricoma and M. ligustroides showed no difference in the germination percentage at the three temperatures tested, but germination delayed at 20 °C. M. radii germinated only at 25 °C and 20 °C. Emergence was slow, both in the greenhouse and in B.O.D, with final germination of M. sellowiana completed by about 70 d in the greenhouse. The temperatures of 25 ºC and 30ºC were found an important factor in the germination of Melastomataceae species in Campos Gerais, with M. Auricoma having initial growth slow.

Keywords: Gibberellic acid; Growth; Seedling establishment; Temperature.

Germinação e emergência de Miconia sp (Melastomataceae)

Melastomataceae é uma das famílias predominantes nos Campos Gerais Paranaenses. No entanto, a germinação e o crescimento inicial da espécie na região têm sido pouco estudados. Para investigar a germinação, emergência e crescimento inicial de cinco espécies de Melastomataceae no Parque Estadual do Guartelá e no Parque Estadual de Vila Velha, foram realizados bioensaios de germinação e testes de emergência a 20 ºC, 25 ºC e 30 ºC em um ambiente de germinação câmara e o crescimento inicial foi avaliado em casa de vegetação. Miconia cinerascens e Miconia sellowiana apresentaram maior porcentagem de germinação (% G) a 25 ºC e 30 ºC. M. auricoma e M. ligustroides não apresentaram diferença na porcentagem de germinação nas três temperaturas testadas, mas a germinação atrasou a 20 °C. M. radii germinou apenas a 25 ºC e 20 ºC. A emergência foi lenta, tanto em casa de vegetação quanto em B.O.D, com a germinação final de M. sellowiana concluída em cerca de 70 dias na casa de vegetação. As temperaturas de 25 ºC e 30 ºC foram consideradas um fator importante na germinação de espécies de Melastomataceae nos Campos Gerais, sendo que M. Auricoma teve crescimento inicial lento.

Palavras-chave: Ácido giberélico; Crescimento; Estabelecimento de mudas; Temperatura.

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INTRODUCTION

Seed germination is a physiological process that begins with imbibition and activates metabolic processes that lead to embryo expansion and radicle protrusion (BEWLEY et al., 2013). It is a complex process that depends on several environmental factors such as temperature, light, water, and oxygen availability.

After germination, the seedling stage is viewed as the most sensitive phase of the plant's life cycle, and seedling growth is mostly regulated by environmental resources and disturbances (BHADOURIA et al., 2016). The way manner seedlings respond to biotic and abiotic stresses is crucial for the conservation, management of plant populations, and ecological restoration.However, studies on the establishment of Melastomataceae seedlings are rare.

In Brazil, Melastomataceae species are found in tropical and subtropical regions and in different types of vegetation (MAIA et al., 2014) and used for forest restoration (BRITO et al., 2017). In the Cerrado and the Atlantic Forest, this family stands out because of its diversity of species, area of occurrence, and endemism (BRITO et al., 2017).

A number of studieshave been carried out on the germination of Melastomataceae species, mainly the ones occurring in areas of Cerrado (CARREIRA et al., 2003; RODRIGUES et al., 2013; SILVEIRA et al., 2013a; SILVEIRA et al., 2013b; OLIVEIRA et al., 2014; RIBEIRO et al., 2016; SANTOS et al., 2017). The flora of the Cerrado is widely studied in Brazil, except in the southern region, which comprises some isolated remnants areas of Cerrado in the state of Paraná (MAIA et al., 2014).

Seeds of Melastomataceae apparently do not have morphological or morphophysiological dormancy (SILVEIRA et al., 2013a), even the layer of lignified cells in the seed coating (BASKIN et al., 2014) offersno resistance to germination (CORTEZ et al., 2008). Despite that, there is a lack of work on the germination capacity of Melastomataceae species found in Campos Gerais.

Studies on germination are important for understanding the establishment of a plant community, but there is a lack of research on germination and regeneration of species of Melastomataceae from Campos Gerais, thus, the present work aims to evaluate seed germination and strategies for establishment of five species of Melastomataceae species in Campos Geraisbioma.

MATERIALS AND METHODS

Collection area

The region of Campos Geraisis located in the central-eastern portion in the state of Paraná. The region has a particular landscape that shelters diverse ecosystemsand has been under several anthropic disturbances caused mainly by farmingwith most preserved areas protected by Conservation Units (ALVES et al., 2019).

The Vila Velha State Park (PEVV) in the municipality of Ponta Grossa (25 ° 15′02 ″ S, 49 ° 59′59 ″ W, 900 m altitude) and the Guartelá State Park (PEG) in the municipality of Tibagi (24 ° 36′55 ″ S, 50 ° 15′37 ″ W, 1030 m altitude) stand out with vegetation composed of Campos Sulinos (Southern Fields) bordered by

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elements of the Atlantic Forest and the Cerradoin the northern limit of the Campos Gerais (MORAES et al., 2015).

Selection and collection of plant material

Five species of Melastomataceae were selected for the study, according to the nomenclature used by Michelangeli et al. (2019): Miconiaauricoma (Spring. Ex Mart.) R. Goldenb., MiconiacinerascensMiq, Miconialigustroides (DC.) Naudin, MiconiaraddiiR. Goldenb., and MiconiasellowianaNaudin (Table 1). These species belong to the Miconieae tribe, which is exclusively neotropical and have the fleshy fruits dispersed by several bird species (MICHELANGELI et al., 2008).

Fruitsamples were collected in areas of the PEG and PEVV parks, taken to the laboratory,placed into plastic bags, and refrigerated at ± 6 °C. Fertile branches of plant material were deposited at the Herbarium of the State University of Maringá (HUEM) and at the Herbarium of Nupélia (HNUP) (Table 1).

Species	Collection Date	Collection Site	Municipality	HerbariumCatalogNumber
Miconiaauricoma (Spring. ex Mart.) R.Goldenb.	23-25/10/2018	Guartelá	Tibagi, PR	HUEM 24931
		State Park		
MiconiacinerascensMig.	03/04/2019	Vila Velha	Ponta Grossa, PR	HNUP 17869
		State Park		
Miconialigustroides (DC.) Naudin.	01-02/04/2019	Guartelá	Tibagi, PR	HNUP 17854
		State Park		
MiconiaraddiiR.Goldenb.	03/04/2019	Vila Velha	Ponta Grossa, PR	HNUP 17864
		State Park		
MiconiasellowianaNaudin.	23-25/10/2018	Guartelá	Tibagi, PR	HUEM 34642
		State Park		

Table 1: Species collected in areas of Vila Velha and Guartelá State Parks.

Laboratory bioassays

Fruits were weighed on an analytical balance to determine the fresh mass and the number of seeds per fruit was counted. Germination tests were carried out for each species.Twenty-five seeds were placed into four9-cm Petri dishes containingtwo filter paper discs moistened with distilled water, totaling 100 seeds per treatment. Petri dishes were kept in germination chambers at different temperatures (20 °C, 25 °C, and 30 °C), 12h photoperiod (12h light and 12h dark), and continuous darkness. For the dark treatment, the dishes were wrapped in aluminum and germination was monitoredunder green light. Water absorbed by seeds and evaporated was replenished when necessary. Germination was checked daily, and the seeds were considered germinated after radicle protrusion (2 mm) (FERREIRA et al., 2004).

In another bioassay, the seeds of each species were treated with 500 mgL⁻¹ gibberellic acid (GA₃) and germination tests were performed as described above, however, the seeds were maintained only at 25 ºC and two light conditions (12h photoperiod and continuous darkness).

After incubation, the viability of non-germinated seeds was determined by the tetrazolium test. The non-germinated seeds were placed into penicillin vialsusing a brush,and 10 ml of 0.1% tetrazolium solution was added. The vials containing the seeds immersed in the tetrazolium solution were kept in the dark for 24 hours. At the end of the incubation period, the seeds were removed from solution and longitudinallycut with a blade. The embryos were examined under a stereomicroscope, and those discolored were considered

unviable (VIEIRA et al., 1994), or the absence of the embryo was recorded. Germination was assessed using meangermination time (MGT), germination speed index (GSI), and germination percentage (% G), according to Maguire (1962) and Ferreira et al. (2004).

Emergence analysis

Emergence tests were carried out for each species in germination chambers and greenhouse. In germination chambers, the seeds were placed in plastic cups (50 mL) containing sand and thesubstrate Fertilizare in the proportion 2:1. Each cup received 25 seeds, totalizing 100 seeds in four replications. The cups were kept at 20 °C, 25 °C, and 30 °C in a 12h photoperiod (12h light and 12h dark) and continuous darkness. In the continuous darkness test, plastic cups were placed inside a cardboard box to block the entry of light and the monitoring of germination was performed in a dark room with green light.

For emergence tests in greenhouse, cells of Styrofoam trays containing sand and organic fertilizer in a 2:1 ratio were sown one seed each, totaling 100 seeds per species. Emergence was monitored daily, recording the total expansion of the first pair of eophiles. The trays were automatically irrigated with watering cycle of 12 hours. The percentage of emergence and emergence speed index of seedlings were calculated using the data of germination tests from the chambers and greenhouse, according to Maguire (1962).

Assessment of initial seedling growth

Evaluation ofinitial growth in greenhouse was carried out only with the seedlings of M. auricoma because of the frost that occurred in Maringá-PR, on the 06, 07, and 08 July 2019, causing the death of most of the seedlings. Of the species maintained in greenhouse, M. auricomahad the highest number of seedlings, allowing the experiment to continue for later evaluation of growth.

Growth was assessed at 140 days after emergence, by randomly choosing 20 seedlings of M. auricoma. The following morphological variables were considered separately: height (H) and root length(RL), dry shoot mass(DSM), and dry root mass (DRM), stem diameter (SD), and leaf number (LN).

Leaf number wasdetermined by counting the fully expanded leaves of each plant. The root length and height was measured with a metric ruler. The collar diameter was measured with a digital caliper. Afterwards, the plants were separated into roots, stems, and leaves and placed into paper bags, labeled, and dried in an oven at 60ºC for 24 hours. The dry mass of the different organs was measured in an analytical balance.

Data analysis

The analyses were always performed to compare the means obtained in the evaluations of the three treatments. The variables with p<0.05 were considered significant. To perform the t-test and ANOVA, the assumptions of independence (in the experimental design) were tested, normality with the Shapiro-Wilk test and homoscedasticity with the Levene test.

The germination data were analyzed by the Tukey's test, with three temperature levels (20 °C, 25 °C, and 30 °C) and one light condition (12-hour photoperiod) using the program Statistica 7.0. The "continuous darkness" treatment was compared with the "12-hour photoperiod" condition only for M. sellowiana and M. auricoma. The emergencedata were analyzed by the t test, using the program Statistica 7.0.

RESULTS

Seeds of M. cinerascens, M. ligustroides, and M. raddii germinated only when exposed to the 12-h photoperiod, whereas the seeds of M. sellowiana and M. auricoma germinated in both continuous darkness and the 12-h photoperiod.

Seeds of M. sellowiana and M. auricomapresented 56% G and 48% G at 25 ºC and 56% G and 18% G at 30 °C, respectively, when kept in continuous darkness, with no significant differencefrom seeds germinated in the 12-h photoperiod. M. sellowiana and M. auricoma seeds also delayed germination (longerMGT) at 25ºC and continuous darkness compared with seeds germinated at 25ºC with a 12-hour photoperiod, that is, the mean time for germination was 17.68 days for *M. sellowiana* and 21.61 days for *M.* auricoma. In relation to GSI, the seeds of M. sellowiana that germinated in "continuous darkness" also presented lower GSI (0.81 and 0.65) at 25 ºC and 30ºC, respectively, when compared with the seeds germinated in the 12-h photoperiod.

Considering only the germination in the 12-h photoperiod, the seeds of M. cinerascens, M. sellowiana, and M. ligustroides showed higher% G and GSI at 25 °C and 30°C, with no difference between these temperatures (Table 2). The MGT was lower for the seeds of M. sellowiana maintained at 25 °C and 30ºC, indicating a faster germination (Table 2).

Species	Temperature (° C)	%G	GSI	MGT (days)
M. auricoma	20	32.00 ± 6.51 a	0.39 ± 0.13 a	$27.43 \pm 3.0 a$
	25	42.00 ± 6.51 a	0.75 ± 0.13 a	11.54 ± 3.0 b
	30	20.00 ± 6.51 a	0.45 ± 0.13 a	11.87 ± 3.0 b
M. cinerascens	20	4.00 ± 7.23 b	0.07 ± 0.16 b	15.13 ± 3.30 a
	25	51.00 ± 7.23 a	0.79 ± 0.16 a	19.15 ± 3.30 a
	30	37.00 ± 7.23 a	0.92 ± 0.16 a	15.19 ± 3.30 a
M. ligustroides	20	44.00 ± 14.71 a	0.35 ± 0.29 a	36.15 ± 2.96 a
	25	64.00 ± 14.71 a	1.15 ± 0.29 a	16.80 ± 2.96 b
	30	37.00 ± 14.71 a	0.79 ± 0.29 a	8.20 ± 2.96 b
M. raddii	25	34.00 ± 2.83 a	0.26 ± 0.02 a	34.10 ± 1.08 a
	30	14.00 ± 2.83 b	0.15 ± 0.02 b	27.88 ± 1.08 b
M. sellowiana	20	49.00 ± 6.24 b	0.53 ± 0.20 b	25.08 ± 1.85 a
	25	87.00 ± 6.24 a	1.77 ± 0.20 a	13.38 ± 1.85 b
	30	86.00 ± 6.24 a	1.81 ± 0.20 a	13.65 ± 1.85 b

Table 2: Meangermination percentage (%G), germination speed index (GSI), and mean germination time (MGT) of Melastomataceae species in a 12-hour photoperiod.

Means followed by the same letter in the column are not significantly different (Tukey, $p < 0.05$). Mean ± standard error.

Seeds of M. cinerascens, M. ligustroides, and M. sellowianaat 20 ºChad %G reduced with high MGT, showing that the seeds germinated slowly.

The different temperatures showed no effect on the %G and GSI of M. auricoma seedsin the 12-h photoperiod (Table 2), however, germination was faster when the seeds were kept at 25 ºC and 30 °C. M. raddii seeds showed higher%G at 25 °C and lower MGT at 30 °C (Table 2).

Figure 1 illustrates the accumulated germination of the five species studied at temperatures of 20 ºC, 25 ºC, and 30 °C. The species in the study showed the highest accumulated germination at 25ºC, noting that M. sellowiana reached the highest % G among the other species, with 80% of the seeds germinating 20 days after the beginning of the incubation (Fig. 1B). M. auricoma and M. raddii showed the highest germination accumulated at 25 °C (Fig. 1B), however, seeds of M. raddii did not germinate at 20 °C (Fig. 1A).

Figure 1: Percentage of accumulated germination of seeds of Miconia species at 20 °C (A), 25 °C (B), and 30 °C (C).

The treatment with gibberellic acid (GA₃) had no promoting effect on the% G or the germination speed of the seeds, that is, there was no significant difference between the seeds treated with GA3 and the untreated seeds (Table 3).

The tetrazolium test showed that seeds of the speciessubjected to the different treatments which fail togerminate were non-viable. The species responded differently in relation to emergence in greenhouse and in B.O.D at 25 °C (Table 4). M. auricoma presented a higher emergence percentage (%E) in greenhouse,

but with a longer time for emergence. However, M. raddiishowed a higher %E, ESI, and MET in B.O.D (Table 4). M. sellowiana and M. ligustroides also presented a higher %E and ESI in B.O.D. However, M. sellowiana showed shorter time for emergence, while M. ligustroidesshowed a higher MET in B.O.D. M. cinerascens did not emerge in greenhouse, showing low % E with high mean emergence time in B.O.D.

Table 3: Germination percentage (% G), germination speed index (GSI), and mean germination time (MGT) of Melastomataceae species treated with gibberellic acid (GA3).

Means followed by the same letter in the column are not significantly different (Tukey, $p < 0.05$). Mean ± standard error.

Table 4: Means of emergence percentage (% E), emergence speed index (ESI), and mean emergence time (MET) of Melastomataceaespecies in a 12-h photoperiod.

Means followed by the same letter in the column are not significantly different (t test, $p < 0.05$). Mean \pm standard error.

Figure 2: Germination and detail of the trichomes in cotyledon leaves and first eophyll, A: Seed at the beginning of germination, B: Beginning of appearance of cotyledon leaves, C: TrichomesM. auricoma (15 days), D: TricomesM. auricoma (140 days), E: TrichomesM. raddii, F: TrichomesM. cinerascens, G: TrichomesM. ligustroides, H: TrichomesM. sellowiana, Scale 5 mm.

No seedling emergence was recorded in the condition of continuous darkness in B.O.D. Considering the initial growth of M. auricoma in greenhouse, it was found that at 140 days after emergence, the plants had on average 0.45 cm of height (H), 1.26 cm of root length (RL), 0.3 mm of stem diameter (SD), and leaf number (LN) ranged from 2 to 7. The dry root mass was 0.35 mg, the dry shoot mass was 0.86 mg, and the total dry mass was 1.2 mg.

The seedlings of the species analyzed, at the time of emergence, haveabundanttrichomes (Fig. 2), which is also observed in *M. auricoma*, at 140 days (Fig. 2D). Germination is epigeal (Fig. 2A and 2B), the seedlings are phanerocotyledonouswith leafy cotyledons (Fig. 2).

DISCUSSION

Light is a fundamental factor for breaking dormancy in photoblastic seeds and is a factor also associated with small seeds, which have limited reserve resources (BEWLEY et al., 2013). The availability of light also enables photosynthesis and continuity of growth of seedlings that arise from the germination of positive photoblastic seeds. Light is also associated with epigeal germination and the presence of leafy cotyledons of Miconiaspecies.

Most of the species in this studyfail to germinate under continuous darkness, which may indicate the positive effect of light on germination (positive photoblastism). Rodrigues et al. (2013) found that Trembleya laniflora seeds germinated only in light. The seeds of Melastomataceae, under controlled conditions, are positively photoblastic and their germination under dark conditions is considered insignificant, regardless of phylogenetic position, geographic distribution, type of growth, or temperature (SILVEIRA et al., 2013a). However, M. auricoma and M. sellowianagerminated even under continuous darkness. Temperature is the second most important environmental factor for seed germination, regulating germination by determining the germination capacity and rate of non-dormant seeds or by removing primary or secondary dormancy (BEWLEY et al., 2013). The temperature-promoting effect on germination can be observed in M. cinerascens, which presented higher% G at higher temperatures (25 °C and 30 °C), as well as the highest GSI.

A low germination percentage associated with a high MGT shows that the seeds germinate slowly, which was found for *M. sellowiana* at 20 °Cand through cumulative germination (Fig. 1A). Moreover, the effect of temperature on germination is important for the ecology of populations. Additionally, in view of the time of obtaining the fruits of M. sellowiana for the experiment (October), the results for the species may be associated with the period of fruit dispersion, which was spring-summer, when temperatures are generally higher. However, M. ligustroides seeds germinated at all temperatures tested, but there was a significant delay in germination (high MGT) when the seeds were kept at 20 °C.

The lack of the promoting effect of gibberellic acid on the germination of the Melastomataceae species studied may be associated with the concentration used or with characteristics of the seed coat. Rodrigues et al. (2010) also found no difference between the treatments with gibberellic acid and the control for the germination percentage inM. ferruginata. Chaves et al. (2011) associated the increased germination of M. ligustroides seeds with the effect of sulfuric acid and subsequent immersion in 400 mg L^1 of GA₃ for 12

hours. The lack of increment in the germination of *Chaetostomaarmatum* (Melastomataceae) seeds treated with GA3 was also reported by Ribeiro et al. (2015). Dormancy breaking with sulfuric acid was not carried out in our study, butthe treatment with GA_3 decreased the germination percentage, reducing the GSI in M. raddii, M. ligustroides, and M. sellowiana.

In the natural environment, Melastomataceae seeds are dispersed by different species of birds and rodents, which could facilitate seed germination after passage through the animals' digestive tract (SILVEIRA et al., 2013a). However, studies on the effect of the zoochoric dispersal syndrome on the germination of Melastomataceae found that the passage of seeds through the birds' intestines, which fed on Melastomataceae, not only did not improve germinationbut also delayed the germination of M. ligustroides (RIBEIRO et al., 2016; SANTOS et al., 2017).

Saatkamp et al. (2019) discuss that the germination time is related to the characteristics of the seeds and to the detection of the ideal regeneration environment through the seed coat permeability, germination requirements, chemical suggestions, and dormancy break requirements. These characteristics of germination and dormancy lead to a germination time that not only prevents unfavorable conditions, but also improves the seedlings' fitness, providing germinated seeds in ideal environmental conditions. This may explain the longer time for emergence required by the species (Table 4), which averagedfrom 30 to 58 days.

Seed dormancy is the main mechanism for controlling the time of seed germination in seasonal ecosystems (BASKIN et al., 2014). Some studies carried out at the community level suggest that seed germination is controlled by both seed dormancy and seed dispersal time. Therefore, the crucial function of dormancy is to prevent germination when conditions are unsuitable for germination and the probability of survival and growth of seedlings is low (FENNER et al., 2005).

Carreira et al. (2003) reported that plants of M. albicans, from seeds collected in the cerrado, at 4 months of growth, were 5 to 15 cm long and had 5 to 10 leaves, that is, approximately the same growth time found in the present work for M. auricoma. These results demonstrate the slow growth of Melastomataceae plants, regardless of the place of origin (Cerrado or Campos Gerais), which may be a particular characteristic of the species. In addition, the low ESI and the high meanemergence time corroborate the indication of the slow growth of the Melastomataceae species studied.

Seedling morphological characteristics may indicate strategies for establishment.This was observed regarding the trichomes in the seedlings of the five Melastomatacea especies, as being a way to avoid excessive transpiration. Furthermore, seedlings of Miconia species showed characteristics related to the requirement for light availability for the growth and performance of metabolic activities such as photosynthesis. However, additional studies are needed to establish the ideal conditions for the growth of these species.

CONCLUSIONS

Conducting this study led to the following conclusions: Miconia species in the region of Campos GeraisParanaensesgerminate better at 25 ºC and 30 ºC, but further studies about environmental factors that may influence seed germination are needed; Only seeds of Miconiaauricoma and Miconiasellowiana germinated at 25ºC in continuous darkness and Miconia radii failed to germinate at 20ºC.;The long time required for emergence of the species and the slow initial growth observed in Miconia auricoma indicate the importance of furthering the studies for the conservation of these species.

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