

# Ecophysiology of seed germination in *Digitaria insularis* ((L.) Fedde)<sup>1</sup>

## Ecofisiologia da germinação de sementes de *Digitaria insularis* ((L.) Fedde)

Giovana Soares de Mendonça<sup>2</sup>, Cibele Chalita Martins<sup>3\*</sup>, Dagoberto Martins<sup>4</sup> e Neumárcio Vilanova da Costa<sup>5</sup>

**ABSTRACT** - The invasive behaviour of sourgrass (*Digitaria insularis*) in cultivated areas is due to its strategy of aggressive regeneration, which is based on seed germination. Knowledge of the physiological ecology of this species can contribute to the development of management and control strategies. The aim of this research was to understand the effects of provenance, temperature and light on the germination of sourgrass seeds collected in the Brazilian state of São Paulo in the cities of Americana, Botucatu and São José do Rio Preto and in the state of Paraná in the city of São Miguel do Iguçu. The seeds were left to germinate at temperatures of 15, 25, 35 and 45 °C, both with and without light. The number of normal seedlings was recorded daily from seven to 60 days. After this period, the seeds together with substrate, were transferred to 25 °C with light, and a daily count was made for all treatments until the end of germination (75 days after sowing). The seeds of *D. insularis* are positively photoblastic. Seed germination in this species depends on provenance. A temperature of 45 °C for germination is lethal to the seeds. The temperature of 35 °C in the presence of light is the most favourable condition for seed germination.

**Key words:** Sourgrass. Plant physiology. Provenance. Light. Temperature.

**RESUMO** - O comportamento invasivo do capim-amargoso (*Digitaria insularis*) em áreas de cultivo deve-se à sua estratégia de regeneração agressiva, que está alicerçada na germinação das sementes. O conhecimento da ecofisiologia dessa espécie pode contribuir para o desenvolvimento de estratégias de manejo e controle. Objetivou-se com a presente pesquisa conhecer o efeito de diferentes procedências, temperaturas e luz na germinação de sementes de capim-amargoso. As sementes foram coletadas no Estado de São Paulo nas cidades de Americana, Botucatu e São José do Rio Preto e no Estado do Paraná na cidade de São Miguel do Iguçu e, colocadas para germinar em temperaturas de 15; 25; 35 e 45 °C, com e sem luz. Foram contabilizadas diariamente as plântulas normais de sete a 60 dias. Após esse período, os substratos com sementes foram transferidos para 25 °C na presença de luz realizando-se contagem diária até que a germinação cessasse em todos os tratamentos (75 dias após a sementeira). As sementes de *D. insularis* são fotoblásticas positivas. A germinação das sementes desta espécie depende da procedência. A temperatura de 45 °C para a germinação é letal às sementes. A temperatura de 35 °C combinada com luz é a condição mais favorável para a germinação das sementes.

**Palavras-chave:** Capim-amargoso. Fisiologia vegetal. Procedência. Luz. Temperatura.

\*Autor para correspondência

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<sup>2</sup>Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", campus de Jaboticabal, Jaboticabal-SP, Brasil, giovana.s.m@hotmail.com

<sup>3</sup>Departamento Produção Vegetal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", campus de Jaboticabal, Jaboticabal-SP, Brasil, cibeled@fcav.unesp.br

<sup>4</sup>Faculdade de Ciências Agronômicas, Universidade Estadual Paulista "Júlio de Mesquita Filho", campus de Botucatu, Botucatu-SP, Brasil, dmartins@fca.unesp.br

<sup>5</sup>Universidade Estadual do Oeste do Paraná/Unioeste, campus de Marechal Cândido Rondon, Marechal Cândido Rondon-PR, Brasil, neumarciovc@hotmail.com

## INTRODUCTION

*Digitaria insularis* (L.) Fedde, known locally as *capim-amargoso*, is an important weed species in the cultivation of coffee and citrus crops. Under Brazilian climatic conditions this species occurs in the field throughout the year, and with the confirmation of the existence of glyphosate-resistant biotypes, the problem has worsened (GEMELLI *et al.*, 2012; MELO *et al.*, 2012).

Knowledge of the ecophysiology of seed germination in weeds such as *capim-amargoso* enables the implementation of studies in the field and in the greenhouse which are aimed at chemical control and the understanding of seed emergence in the soil, besides providing guidelines for the adoption of management practices that are unfavourable to this emergence (GEMELLI *et al.*, 2012; MARTINS; PEREIRA; MARCHI, 2011; MARTINS; MARTINS, 2013).

Germination can be affected by the intrinsic conditions of the seed, such as viability and dormancy, and by environmental factors, such as the temperature and the availability of water, oxygen and light (OAK; NAKAGAWA, 2012).

Light is necessary for the germination of some species of weed, but is not considered a basic factor for non-dormant seeds (MARTINS *et al.*, 2000). Seeds are classified according to their response to light as: positively photoblastic, whose ability to germinate increases in the presence of light; negatively photoblastic, which germinate in the absence of light; and neutrally photoblastic, where germination is not affected by light (OAK; NAKAGAWA, 2012).

Various species of *Digitaria* show different responses to the presence or absence of light for seed germination (KLEIN; FELIPPE, 1991; MONDO *et al.*, 2010). According to Klein and Felipe (1991), seeds of *D. horizontalis* are positively photoblastic and those of *D. insularis* are neutral.

Although seed germination may take place over wide temperature ranges, there are certain temperatures that enable the maximum speed and uniformity of the process, the so-called optimal temperatures. There are also maximum and minimum temperatures, above and below which no germination occurs (OAK; NAKAGAWA, 2012).

The percentage of germination in *D. insularis* is relatively high (50%) compared to other species of the same genus, such as *D. bicornis* (Lam.) Roem. and Schult., *D. ciliaris* (Retz.) Koel, and *D. horizontalis* Willd (MONDO *et al.*, 2010). In these four species a higher percentage and speed of germination were seen under alternating temperatures than under constant temperatures; however that study was conducted with seeds from a single location only, with the requirements of temperature and light being dependent on the species.

The emergence of ecotypes adapted to different conditions of soil and climate is a factor of concern in weed research, as species tend to invade and settle in new areas (BIANCO; BARBOSA JUNIOR; PITELLI, 2004). In the literature there are reports of ecotypes of *D. insularis* with varying growth patterns, aggressiveness and tolerance to herbicides (GEMELLI *et al.*, 2012; MELO *et al.*, 2012).

These ecotypes originate with phenotypic changes that ensure propagation and survival at sites having different conditions of soil, climate and management, thereby contributing to the ecological and evolutionary success of the plant (PITELLI; BIANCO, 2013). The place of origin of the seeds may therefore influence their germination and vigour (BOGNOUNOU *et al.*, 2010; MARTINS *et al.*, 2000).

The aim of this work was to evaluate the effect of different sources, temperature and light on the germination of seeds of *D. insularis*.

## MATERIAL AND METHODS

Seeds of *D. insularis* were collected in July 2012 in the municipalities of Americana, Botucatu and São José do Rio Preto in the state of São Paulo (SP), and in São Miguel do Iguaçu in the state of Paraná (Figure 1), whose climatic conditions and geographical coordinates are shown in Table 1.

Once harvested, seeds from the four places of origin were taken to the Laboratory of Seed Analysis of the Department of Plant Production at the São Paulo State University (UNESP), Jaboticabal, SP, where they were stored in a dry chamber (45% RH and  $\pm 20$  °C) for approximately one month until the tests were set up.

**Figure 1** - The states of São Paulo and Paraná in Brazil, showing the location of the cities of origin of the seeds



**Table 1** - Geographical and climatological information of the origins of the seeds of *D. insularis* used in the trial

Locality**	Climate*	Average annual temperature			Latitude	Longitude	Altitude (m)	Annual rainfall (mm)
		Mín.	Ave.	Máx.				
SJRP	Aw	17.0	23.6	30.4	20°49'12"S	49°22'44" W Gr.	500	1,259
BOT	Aw	15.3	20.7	26.1	22°53'09"S	48°26'42" W Gr.	804	1,359
AME	Cwa	15.3	21.8	28.2	22°44'20"S	47°19'51" W Gr.	560	1,292
SMI	Cfa	16.7	21.4	27.6	25°26'00"S	54° 22'00" W Gr.	260	1,831

\*Köppen climate classification. Sources: CEPAGRI - Centro de Pesquisas Meteorológicas e Climáticas Aplicadas à Agricultura (2013). IAPAR - Instituto Agrônomo do Paraná (2013); (\*\*) SJRP (São José do Rio Preto), BOT (Botucatu), AME (Americana) and SMI (São Miguel do Iguçu)

The germination test was carried out with four replications of 50 seeds, under constant temperatures of 15, 25, 35 and 45 °C, on two sheets of germination paper moistened with 12 mL of distilled water, inside plastic boxes (11.0 x 11.0 x 3.5cm), both with and without light. For the tests with light, transparent boxes were used at a photoperiod of eight hours; for germination in the dark, black plastic boxes were employed, with the germination counts taken in a dark room under green light.

In evaluating germination, normal germinated seedlings were considered, with a daily count of those with primary root protrusion and well-developed shoots being taken from seven to sixty days after sowing. The first germination count test was performed seven days after sowing. The results were expressed as percentages.

After the period planned for the germination test, the boxes containing seeds that had not germinated were transferred to a chamber set to 25 °C under light, these being excellent conditions for most cultivated species (MARCOS FILHO, 2005). After transfer, daily counts were taken, again for all treatments, until germination ceased, when the seeds that had germinated were then counted.

The total percentage of viable (live) seeds was calculated at 75 days based on the sum of germinated seeds (germination at the initial temperature plus germination at 25 °C with light) for the total test period.

The experimental design was completely randomised, in a 4 x 4 x 2 factorial scheme, where the treatments comprised a combination of the four places of origin of the seeds (Americana, Botucatu, São José do Rio Preto and São Miguel do Iguçu), four germination temperatures (15, 25, 35 and 45 °C) and two light conditions (with and without light), with four replications. Whenever the result of the variance analysis was significant at 5%, the averages for the treatments were compared by Tukey test at 5% probability.

## RESULTS AND DISCUSSION

The summary of the variance analysis for first count and viability showed interactions between origin

and temperature, origin and light, and temperature and light. For the parameter germination, a significant effect was seen only from the interaction between temperature and light at 1% probability (Table 2).

In the interaction of origin and temperature (Table 3), an effect was seen for temperature on first germination count for all places of origin. The first temperature of 35 °C was the most favourable, followed by 25 °C. At 15 and 45 °C there was no germination, independent of origin, as evaluated by the first count test.

As the temperature reduces from the optimal to the minimum, there is a reduction in the rate of germination; this is due to such conditions not favouring the process of imbibition, the enzymatic activity (BHATTACHARJEE; MUKHERJEE, 1995) and consequently, the mobilisation of reserves.

In evaluating the effect of origin, seeds from São José do Rio Preto and Botucatu were superior at a temperature of 25 °C for rate of germination evaluated by the first count test, compared to those from Americana and San Miguel do Iguçu. At 35 °C, the seeds from Americana in the first count test germinated at a higher percentage than did the others; with the seeds from São José do Rio Preto resulting in the lowest values. Seeds from Botucatu and São Miguel do Iguçu showed intermediate values when compared to seeds from the other places of origin (Table 3).

From the averages of the interaction between origin and light, it was found that seed germination was stimulated or not by the presence of light depending on the origin, since seeds collected in São José do Rio Preto and Americana expressed higher percentages for germination in the presence of light, while those collected in Botucatu and São Miguel do Iguçu were not affected; the first germination count of seeds from these towns being similar under both light conditions (Table 4).

Different responses as regards the acceleration of germination in the presence of light may be related to the evolutionary adaptation of the species to the place of origin (BASKIN; BASKIN, 1998; MARTINS *et al.*, 2000; MARTINS *et al.*, 2013).

**Table 2** - Variance analysis of the effect of origin, temperature and light for the tests of first count, germination and viability in seeds of *Digitaria insularis*

Cause of variation	GL	Test		
		First Count	Germination	Viability
Origin (O)	3	9.17**	17.00**	45.75**
Temperature (T)	3	638.40**	599.34**	709.17**
Light (L)	1	66.42**	372.52**	74.33**
(O) x (T)	9	30.58**	3.96 <sup>ns</sup>	21.42**
(O) x (L)	3	13.70**	2.18 <sup>ns</sup>	17.66**
(T) x (L)	3	26.05**	67.65**	11.60**
(O) x (T) x (L)	9	7.67**	4.82**	20.05**
QM (Residual)	96	23.2	51.6	42.3
C.V (%)	-	34.4	23.4	14.7

\*\* : significant at 1% probability; <sup>ns</sup>: not significant; MS = mean square

**Table 3** - First germination count data (%) in seeds of *D. insularis* from the breakdown of the interaction between origin and temperature

Temperature (°C)	Origin			
	São José do Rio Preto	Botucatu	Americana	São Miguel do Iguçu
15	0 aC	0 aC	0 aC	0 aC
25	17 aB	17 aB	6 bB	3 bB
35	29 cA	45 bA	66 aA	42 bA
45	0 aC	0 aC	0 aC	0 aC
d.m.s (Origin)	6.3			
d.m.s (Temperature)	6.3			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

**Table 4** - First germination count data (%) in seeds of *D. insularis* from the breakdown of the interaction between light and origin

Light	Origin			
	São José do Rio Preto	Botucatu	Americana	São Miguel do Iguçu
With	19 aA	14 bA	18 abA	20 aA
Without	5 cB	11 bA	10 bB	17 aA
d.m.s (Origin)	3.4			
d.m.s (Light)	4.5			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

In relation to the effect of the interaction of light and temperature evaluated by the first germination count test (Table 5), it was found for both light conditions, that the temperature of 35 °C was the most favourable, followed by 25 °C. There was however no difference

between the conditions of 25 °C in the absence of light and the two most unfavourable conditions of 15 and 45 °C. Seeds kept at 15 and 45 °C did not germinate until the seventh day, when the first count was taken for both light conditions.

**Table 5** - First germination count data (%) in seeds of *D. insularis* from the breakdown of the interaction between light and temperature

Light	Temperature (°C)			
	15	25	35	45
With	0 cA	20 bA	50 aA	0 cA
Without	0 bA	2 bB	41 aB	0 bA
d.m.s (Temperature)	3.4			
d.m.s (Light)	4.5			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

In relation to the evaluation of germination for the interaction between temperature and origin, 35 °C was the most favourable temperature for seed germination, followed by 25 and 15 °C for all places of origin except São José do Rio Preto (Table 6). The worst temperature was 45 °C which, with the exception of Americana, precluded germination. These results are similar to those observed in Table 3 for vigour evaluated by first count.

As the temperature rises from optimal to maximum, there is a decrease in oxygen availability and enzyme activity and in the synthesis of RNA, DNA, sugars and ATP (PIROVANO *et al.*, 1997; STEWART *et al.*, 1990), culminating in the coagulation of enzymes and in the deterioration and death of the seeds (SMITH; SHAW; NEWSON, 1992).

At temperatures of 25 and 35 °C, a greater percentage of germination was seen in the seeds from Americana and São José do Rio Preto than in those from Botucatu; these in turn did not differ in germination from those from São Miguel do Iguacu. Overall, at 15 °C, seeds from Botucatu and São José do Rio Preto displayed intermediate values for germination compared to those from Americana and São Miguel do

Iguaçu, which had a higher and lower percentage of germination respectively.

The interaction between origin and light on the germination of the seeds showed that the presence of light was a determining factor in promoting a greater percentage of seed germination for all places of origin. The species therefore produces seeds which are positively photoblastic (Table 7). These results differ from those obtained by Klein and Felipe (1991), who classified the seeds of this species as being indifferent to light. However, those researchers were evaluating seeds from only one area, Piracicaba, SP.

In the presence of light, seeds harvested in both Americana and São José do Rio Preto displayed similar percentages for germination, greater than for the other places of origin where there were no differences in germination. In the absence of light, seeds from the town of Americana displayed the highest values for germination, higher than those from Botucatu or São Miguel do Iguacu (Table 7). The seeds from São José do Rio Preto displayed intermediate results for germination values, and did not differ from the other places of origin.

Among the temperatures that were evaluated, 35 °C was the most favourable to germination under

**Table 6** - Germination data (%) in seeds of *D. insularis* from the breakdown of the interaction between origin and temperature

Temperature (°C)	Origin			
	São José do Rio Preto	Botucatu	Americana	São Miguel do Iguacu
15	21 abC	20 abB	29 aB	19 bB
25	33 aB	16 cB	29 abB	24 bcB
35	82 aA	71 bA	83 aA	62 bA
45	0 aD	0 aC	4 aC	0 aC
d.m.s (Origin)	9.4			
d.m.s (Temperature)	9.4			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

**Table 7** - Germination data (%) in seeds of *D. insularis* from the breakdown of the interaction between light and origin

Light	Origin			
	São José do Rio Preto	Botucatu	Americana	São Miguel do Iguaçu
With	50 aA	37 bA	48 aA	38 bA
Without	19 abB	16 bB	25 aB	15 bB
d.m.s (Origin)	6.6			
d.m.s (Light)	5.0			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

both light conditions. The temperatures of 15 and 25 °C displayed intermediate behaviour and were similar to each other, although without light, the temperature of 45 °C showed no difference to either (Table 8). The temperature of 45 °C did not result in seed germination.

As for the interaction between temperature and origin on viability, it was found that the towns of Americana and Botucatu produced seeds that displayed higher viability at 15 °C than those from São Miguel do Iguaçu and São José do Rio Preto (Table 9).

The superior performance of Americana and Botucatu can be attributed to the dormancy induced by the extreme temperatures which are unfavourable to the species, and could perhaps be a defence mechanism which is able to keep the seeds viable when exposed to adverse conditions (CARVALHO; NAKAGAWA, 2012; MARTINS *et al.*, 2013). Once transferred to the optimal conditions of 25 °C, these seeds would come out of this state of dormancy (Figure 2) in a similar way to that observed for seeds of the *melaleuca*, also a weed, which is found on waterlogged land (MARTINS *et al.*, 2013).

**Table 8** - Germination data (%) in seeds of *D. insularis* from the breakdown of the interaction between light and temperature

Light	Temperature (°C)			
	15	25	35	45
With	42 bA	47 bA	84 aA	0 cA
Without	2 bB	4 bB	66 aB	2 bA
d.m.s (Temperature)	6.6			
d.m.s (Light)	5.0			

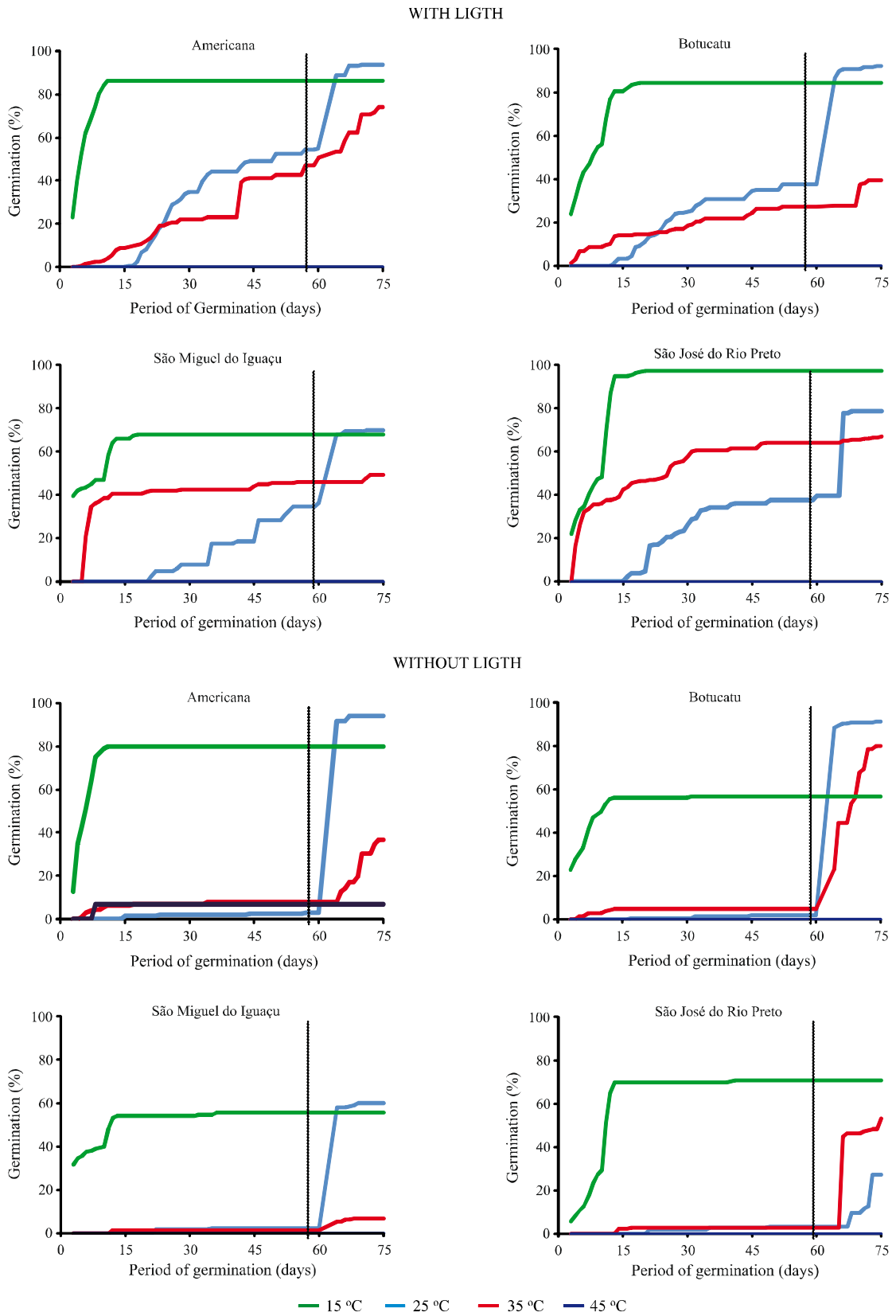
Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

**Table 9** - Viability data (%) in seeds of *D. insularis* from the breakdown of the interaction between origin and temperature

Temperature (°C)	Origin			
	São José do Rio Preto	Botucatu	Americana	São Miguel do Iguaçu
15	53 cB	92 aA	94 aA	65 bA
25	60 aB	60 aC	56 aC	58 aB
35	84 aA	71 bB	83 aB	62 bA
45	0 aC	0 aD	0 aD	0 aC
d.m.s (Origin)	9.8			
d.m.s (Temperature)	9.8			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

**Figure 2** - Germination (%) in seeds of *capim-amargoso* (*Digitaria insularis* (L.) Fedde) over time, in the presence and absence of light at different temperatures, and after 60 days when they were transferred to conditions of 25 °C with light



It is also noteworthy that the two sites record minimum annual average temperatures lower than those of São José do Rio Preto and São Miguel do Iguacu (Table 1); climatic conditions may therefore have contributed to the selection of individuals adapted to this environment.

At 25 °C seeds from the four different places of origin germinated in a similar way to each other (Table 9). At 35 °C the seeds from São José do Rio Preto and Americana displayed greater viability when compared to the others. Among the places of origin evaluated in this work, it can be noted that these were the towns with the highest annual average and maximum temperatures (Table 1). At these places therefore, the plants must have undergone a selection process for the adaptation and production of seeds that would germinate and remain viable at high temperatures (OLIVEIRA *et al.*, 2014). At 45 °C germination was not observed for seeds from any of the places of origin (Table 9).

In the interaction between origin and light on seed viability, the presence of light favoured viability in the seeds from Americana, showing higher values similar to those seen in seeds from São José do Rio Preto. However, the seeds from São José do Rio Preto did not differ from those from Botucatu. In the seeds from São Miguel do Iguacu, lower viability was seen under both light conditions (Table 10). In the absence of light, the seeds

from Americana and Botucatu behaved in a similar and superior way to those from the other places of origin.

As regards the interaction between temperature and light on the viability of seeds of *capim-amargoso*, it was found that for both light conditions the temperature of 45 °C was lethal to the seeds in a similar way to that observed for seeds of other species of weed, such as *leiteiro* and *melaleuca* (MARTINS *et al.*, 2000; MARTINS *et al.*, 2013). High temperatures may therefore have detrimental effects on the germination of seeds (OLIVEIRA *et al.*, 2014). The temperature of 25 °C resulted in intermediate values for seed viability under both light conditions (Table 11).

For the remaining temperatures, viability in the germination test benefitted from the presence of light. The temperatures of 15 and 35 °C were the most favourable to viability, with similar results.

Under both light conditions and for all places of origin, the temperature of 35 °C resulted in maximum germination before 15 days (Figure 2). Therefore, 35 °C can be considered the optimum temperature for the species (CARVALHO; NAKAGAWA, 2012; MARTINS *et al.*, 2000; MARTINS *et al.*, 2013).

The temperatures of 15 and 25 °C in the presence of light, made a gradual and progressive germination

**Table 10** - Viability data (%) in seeds of *D. insularis* from the breakdown of the interaction between light and origin

Light	-----Origin-----			
	São José do Rio Preto	Botucatu	Americana	São Miguel do Iguacu
With	61 aA	54 bA	64 aA	47 cA
Without	38 bB	57 aA	55 aB	31 cB
d.m.s (Origin)	5.2			
d.m.s (Light)	6.9			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference

**Table 11** - Viability (%) in seeds of *D. insularis* from the breakdown of the interaction between light and temperature

Light	-----Temperature (°C)-----			
	15	25	35	45
With	84 aA	58 bA	84 aA	0 cA
Without	68 aB	44 bB	66 aB	0 cA
d.m.s (Temperature)	6.9			
d.m.s (Light)	5.2			

Averages followed by the same letter, lowercase on a line and uppercase in a column, do not differ statistically by Tukey test at 5% probability; m.s.d = minimum significant difference



of the seeds possible over time. However, with the tests transferred to the condition of 25 °C with light at 60 days, only those seeds which had been kept at 15 °C showed a significant increase in germination, reaching values which were similar to or higher than those for the optimum temperature of 35 °C for Americana, Botucatu and São Miguel do Iguçu (Figure 2).

Under the condition without light, the temperatures of 15 and 25 °C displayed a germination of less than 5% (Figure 2 and Table 8), indicating that at temperatures which are different to the optimal, germination is inhibited. Only after the transfer of the tests at 60 days to the conditions of 25 °C with light, did the seeds display a significant increase in germination. After this transfer, the seeds which had been subjected to a temperature of 15 °C germinated at a higher or similar percentage to that obtained at the optimal temperature, except those from São José do Rio Preto. The seeds that were initially at 25 °C in the absence of light also displayed an increase in germination when transferred to 25 °C with light; however, less evident than that observed for the other evaluated temperatures for most of the places of origin.

It can therefore be said that in general the germinative behaviour of seeds varies according to their place of origin. These results agree with reports in the literature on the existence of ecotypes and differences in germinative behaviour between weed populations (BASKIN, BASKIN, 1998; MELO, 2012).

It can be inferred that the temperatures of 15 and 25 °C in the presence of light made a gradual and progressive germination of *D. insularis* possible over time, however with the tests being transferred at 60 days to the conditions of 25 °C with light, only seeds that had been at 15 °C displayed greater germination, both in the presence and absence of light, except for seeds from São José do Rio Preto. The temperature of 35 °C can be considered the optimum temperature for the species, while the temperature of 45 °C was lethal.

## CONCLUSIONS

1. Seed germination in *D. insularis* depends on origin;
2. The seeds of this species are positively photoblastic;
3. A temperature of 45 °C for germination is lethal to the seeds;
4. A temperature of 35 °C combined with light is the most favourable condition for seed germination.

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