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CROP PROTECTION

Temperature, osmotic stress and light modulating germination of herbicide-resistant and susceptible biotypes of *Amaranthus hybridus* L.

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ABSTRACT: Smooth pigweed is one of the main weed in soybean systems. Knowing the environmental factors that interfere with the germination of the species is important for adapting management practices. The main purpose of this research is to characterize the germination of smooth pigweed biotypes (resistant and susceptible to herbicides) in response to environmental requirements. The trials were carried out in a randomized experimental design with four replications and in a factorial structure, with factor A consisting of herbicide-susceptible and multiple herbicide-resistant and biotypes (ALS and EPSPs inhibitors) and factor B consisting of the different treatments of the environmental factors: the influence of photoperiod, temperature, osmotic potential, and light quality on the germination of the biotypes. Overall, no differences were observed between the tested biotypes in response to the environmental factors evaluated, indicating that resistance did not impose an adaptive cost. The smooth pigweed showed the neutral photoblastic characteristics, with light an additive factor. The temperature range for germination was between 20 and 35 °C, with the best temperature around 30 °C. Germination is reduced due to water stress less than -2.0 Bar. However, at -1.0 Bar, 19% of germination occurs, proving the survival characteristic of this species. The environmental conditions favorable to the germination of smooth pigweed are observed in the same months as the soybean crop cycle in Southern Brazil, and this is the time to intensify monitoring and management.
Key words: *Amaranthus hybridus* L., light quality, photoperiod, water stress, cardinal temperatures.

Temperatura, estresse osmótico e luz como moduladores da germinação em biótipos de *Amaranthus hybridus* L. resistente e suscetível a herbicidas

RESUMO: O caruru-roxo é uma das principais plantas daninhas do sistema de produção da soja e conhecer os fatores ambientais que interferem na e germinação da espécie é importante para adequação das práticas de manejo nas lavouras. O principal objetivo desta pesquisa é caracterizar a germinação de biótipos de caruru-roxo (resistentes e suscetíveis a herbicidas) em resposta às exigências ambientais. Para isso, foram realizados experimentos, em delineamento experimental completamente casualizado com quatro repetições, esquema fatorial, cujo fator A foram os biótipos suscetível e com resistência múltipla (inibidores de ALS e EPSPs), e o fator B os diferentes níveis de condições ambientais, sendo elas: influência do fotoperíodo, temperatura, potencial osmótico e qualidade de luz na germinação dos biótipos. Os resultados da pesquisa indicam que não foram identificadas diferenças entre os biótipos testados em resposta aos fatores ambientais testados, indicando que a resistência não promoveu custo adaptativo. Além disso, o caruru-roxo apresentou características de sementes fotoblástica neutra, com a luz um fator aditivo e a faixa de temperatura para germinação entre 20 e 35 °C, sendo a ótima de 30 °C. A germinação é reduzida sob deficiência hídrica em níveis inferior a -2,0 Bar. Entretanto, a -1,0 Bar, ocorre 19%, de germinação, comprovando a característica de sobrevivência da espécie. Portanto, condições ambientais favoráveis à germinação de caruru-roxo são observadas nos meses que coincidem com o ciclo da cultura da soja para o Sul do Brasil, sendo esse o período para intensificar o monitoramento e manejo.
Palavras-chave: *Amaranthus hybridus* L., qualidade da luz, fotoperíodo, estresse hídrico, temperaturas cardeais.

INTRODUCTION

Smooth pigweed (*Amaranthus hybridus* L.) is one of the main hard-to-kill weeds in soybean system (AVILA NETO et al., 2025). It is a summer annual herbaceous plant that only reproduces by seeds, native to the tropical regions of America, but is currently found worldwide (CABI, 2021). Smooth pigweed is considered a highly competitive weed because of its rapid growth and prolific seed production, characteristics commonly associated with troublesome *Amaranthus* species (BRAZ & TAKANO, 2022). The smooth pigweed species can produce up to 100,000 seeds per plant, and the germination rate can exceed 75% without using methods to eliminate the seeds' dormancy (CABI, 2021; XAVIER et al., 2019).

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Environmental factors can interfere with the germination of smooth pigweed therefore, literature has a divergence regarding the response to photoperiod. Some authors have classified smooth pigweed as positively photoblastic, in other words, the seed depends on the incidence of light to start the germination process (MARTINS et al., 2020). However, there are reports that the presence of light can be additive in the germination process. When light does not alter the germination response, its effect can be considered additive, as seed germination occurs regardless of illumination, although seedling establishment may occur under more favorable physiological conditions in the presence of light (WEI et al., 2023).

Studies using air temperature alternation during the day and night of 20 ºC and 35 ºC, respectively, and a day duration of 16 hours, showed that the smooth pigweed species had germination rates of over 70%. When subjected to constant air temperature conditions of 25 ºC and without lighting, the species showed germination of only 36% (CARVALHO & CRISTOFFOLETI, 2007). However, few studies have examined whether herbicide resistance in this species is associated with fitness costs expressed during seed germination, particularly through changes in cardinal temperatures. Also, there are few reports in the literature on the response of germination to soil osmotic potential and light quality for this species.

For *A. retroflexus* L. the osmotic potential close to -3.5 Bar reduced seed germination by 50% (AHMADVAND et al., 2018), while for *A. palmeri* S. Watson the potential of -3.2 Bar inhibited germination (HAN et al., 2022). Thus, by knowing the environmental factors that interfere with the germination of the species, it is possible to predict the most favorable times for the germination of the species and thus carry out management practices aimed to reduce the spread of the species in crops. Herbicide resistance can influence seed germination and alter the environmental conditions required for germination, potentially imposing a fitness cost on resistant populations. The ACCase-resistant biotypes of *Avena ludoviciana* L. carrying the Ile-1781-Leu mutation have been shown to require higher soil water availability to initiate germination and to germinate more slowly than susceptible biotypes (BENAKASHANI et al., 2021). However, it remains unclear whether multiple herbicide resistance in smooth pigweed affects seed germination responses to environmental factors that regulate germination. Therefore, this research characterized germination and determine the optimum, minimum and maximum thresholds of *A. hybridus* biotypes resistant and susceptible to herbicides in response to temperature, photoperiod, osmotic potential and light quality.

MATERIALS AND METHODS

Five laboratory experiments were carried out at the Department of Crop Protection at the Universidade Federal de Santa Maria in Santa Maria, Rio Grande do Sul (RS) (latitude -29.72°S, longitude 53.72°W and altitude 89 m) from October 2021 to August 2023. The experiments were conducted with 2 biotypes of smooth pigweed, the first coded as 'RS1', proven to have multiple resistance to herbicides inhibiting acetolactate synthase (ALS) and 5-enolpyruvylchiquimate 3-phosphate synthase (EPSPs) from the F2 generation, and the second biotype coded as 'RS2', proven to be susceptible to herbicides of the aforementioned mechanisms of action. The growth and development conditions of the seed parent plants were similar to each other. These biotypes came from the seed bank of Syngenta Proteção de Cultivos - Brazil and exhibit resistance to glyphosate, chlorimuron, and imazethapyr. Resistance to ALS-inhibiting herbicides is conferred by the Trp-574-Leu substitution in the ALS gene, whereas glyphosate resistance is associated with multiple amino acid substitutions (TAP-IVS) in the EPSPS gene (OLIVEIRA et al., 2024).

The experiments were conducted in a factorial scheme, with the exception being the seed characterization experiment, where the only treatment factor was the biotypes. The Factor A always testing two biotypes, and Factor B of each experiment varied according to the simulation of different environmental conditions for weed germination. For all the experiments, a completely randomized experimental design was adopted, with four replications conducted in a growth chamber equipped with four LEDs for lighting (30µmol m^{-2} s^{-1}) with air temperature and photoperiod regulation.

It was not necessary to implement methods to overcome seed dormancy. In addition, the germination tests were conducted in accordance with the methodology proposed for the species, using blotting paper as the substrate (BRASIL, 2009). The experimental units consisted of transparent acrylic gerboxes (11.0 x 11.0 x 3.5 cm), made up of 2 sheets of blotting paper moistened with distilled water in an amount equivalent to 2.5 times the mass of the dry paper. 50 seeds of each biotype were distributed evenly in each gerbox. Supplementary irrigation with distilled water was carried out when necessary.

The first experiment, to initially characterize the seeds germination and the dormancy of the both biotypes was conducted using a 12-hour photoperiod and a constant air temperature of 30 °C. The second experiment to determine the influence of photoperiod on seed germination used a constant air temperature of 30 °C and seven photoperiod levels: 0, 4, 8, 12, 16, 20 and 24 hours. The third experiment on the influence of air temperature, an 8-hour photoperiod and eight levels of air temperature were used: 10, 15, 20, 25, 30, 35, 40 and 45 °C. The fourth experiment, to simulate water stress, the methodology used polyethylene glycol 6000 (PEG) to obtain different osmotic potentials (Ψo). Thus, 0, 35.4, 55.8, 85.4, 127.8, 188.2, 234.4 and 273.9 g of PEG 6000 per kilogram of distilled water were dissolved to obtain osmotic potentials of 0, -0.25, -0.5, -1, -2, -4, -6 and -8 Bar, respectively (MAHMOOD et al., 2016). In this study, a photoperiod of 8 h and a constant air temperature of 30 °C were used. Although alternating temperatures of 20/30 °C are recommended for germination tests of this species (BRASIL, 2009), constant temperature conditions were used to allow the accurate estimation of germination ecophysiological parameters and to isolate the effects of the environmental factors evaluated.

The fifth experiment, to determine the influence of light quality on germination, a photoperiod of 8 hours and a constant air temperature of 30 °C were used in the experiment. In this study, the treatments consisted of 6 levels of simulated wavelengths using different colors as filters (blockers) of the wavelengths: no light, blue (450 nm), green (500 nm), red (700 nm), far-red (760 nm) and white light (380 to 760 nm). To obtain the different qualities of light (blue, green and red), the gerboxes were wrapped in two sheets of cellophane paper with colors corresponding to the treatment used, according to a methodology adapted from YAMASHITA et al. (2008). Thus, for the far-red light treatment, the boxes were wrapped in two sheets of red and blue cellophane, respectively. In the treatment with white light, the gerboxes were not wrapped in cellophane, and the treatment without light was obtained by wrapping the boxes in two layers of aluminum foil. During the daily counting evaluations, all the experimental units were taken to an environment completely free of natural light, illuminated only by green light so as not to interfere with the treatments.

The variable obtained was seed germination, which was monitored daily for up to 14 days after sowing (DAI), until no further germination occurred. A seed was considered germinated when it produced a root or shoot measuring more than 0.1 cm (Figure 1A). In the experiment on the influence of photoperiod on germination, germination was counted at 7 DAI (first count) and 14 DAI (final count), and the seedlings were separated into normal and abnormal. The criteria used to characterize abnormal plants was the presence of symptoms such as epinasty, deformation and other anomalies (Figure 1C).

After the evaluations were completed, the daily count data was transformed into germination percentage according to equation 1.

$$y = (x * 100/z) \quad (1)$$

where y is the germination percentage; x is the number of germinated plants in each experimental unit and z is the number of seeds distributed in each experimental unit.

The results of seed germination percentage were submitted to analysis of variance by the F test. For the second experiment, when statistical significance was found, the means of each treatment were compared using the Scott-Knott test ($P \leq 0.05$). Regression analysis was carried out using the R software (R CORE TEAM, 2012), using the DRC package (RITZ et al., 2015). The Weibull logistic

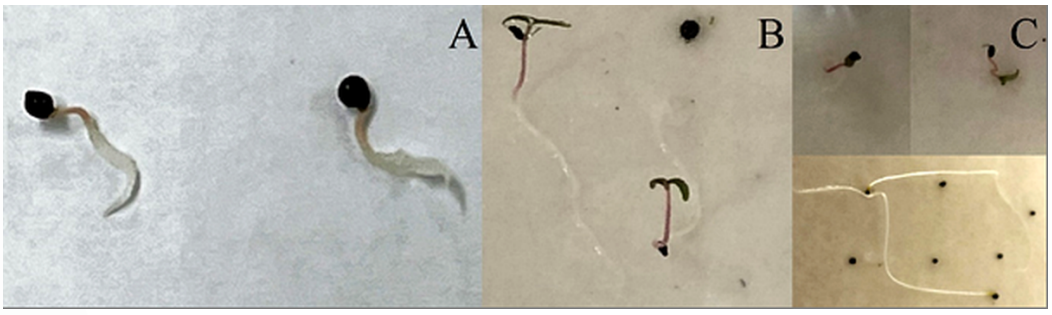


Figure 1 - *Amaranthus hybridus* L. seedlings showing the hypocotyl and radicle, representing the criteria for counting germinated seeds (A) and characterizing normal (B) and abnormal (C) plants.

function with 4 parameters was adjusted for the third, fourth and fifth, as shown in equation 2.

$$y = a + (a-c)\left[1-\exp^{-\left(x-+\frac{x}{d}\right)b}\right] \quad (2)$$

where: *y* is the germination percentage; *x* is the time expressed in days; *a*, *b*, *c* and *d* are empirically derived constants, where *a* in the intercept or the lower limit, *b* is the rate slope, *c* is the upper limit, *d* is the shape parameter. The T_{50} (the time needed to obtain 50% total germination of each treatment) as estimated by the ED function on DRC package.

With the data on the daily accumulation of germinated seeds, we adjusted a linear regression (equation 3) to (1) find the base (Tb), optimum (To) and maximum (Tm) air temperature ranges for the third experiment and (2) find the basal osmotic potential (Ψb) in the fourth experiment as shown as:

$$y = a + bx \quad (3)$$

where for the third experiment the Tb was found of the intercept value, To was found when the two regressions touch each other, Tm was found in the when the regression touch the *x* axis. For the fifth experiment: *y* is the $1/T_{50}$ for temperature ranges; *x* is the temperature (ºC). For the fourth experiment: *y* is the $1/T_{50}$ for osmotic potential levels (Ψo); *x* is the osmotic potential levels (Ψo); *a* is the intercept.

The inverse of T_{50} ($1/T_{50}$) and two independent linear regressions (sub-optimal and supra-optimal) were used to estimate cardinal temperatures (base, optimal and maximum). The optimum temperature (To) was defined at the point where the two lines intersect, and the point where the lines intersect the Xm axis was considered the base temperature (Tb) and maximum temperature (Tm) (DUMUR et al., 1990). To estimate the base osmotic potential (Ψb), the inverse of T_{50} ($1/T_{50}$) was used to generate the regression line. Determining the base osmotic potential (Ψb) at the point of interception of the X axis o (HARDEGREE E EMMERICH, 1994).

RESULTS AND DISCUSSION

Initial characterization of biotype germination

In the initial characterization of the RS1 and RS2 biotypes, the results were submitted to analysis of variance using the F test and were not significant ($P \leq 0.05$). The germination percentages were 32 and 40% at 7 DAI and 40 and 45% at 14 DAI for RS1 and RS2, respectively (data not shown). A germination percentage of around 50% was found for *A. hybridus* under constant temperature conditions of 25 °C and 8 h of light (CARVALHO & CHRISTOFFOLETI, 2007). Therefore, direct comparisons should consider the influence of temperature variation on germination.

No significant differences were observed between the biotypes, indicating that the seed samples used were suitable and representative for the species. The following studies were carried out without the application of additional methods to overcome dormancy and promote uniform germination.

Photoperiod influence on germination

In the study of the influence of photoperiod on germination, the ability of both biotypes to germinate was observed in all treatments, showing statistical differences in the Scott-Knott test ($P \leq 0.05$) for factor B (photoperiod) at 14 DAI, with no significant effect for factor A (biotypes) and no interaction between the factors (Table 1). The total germination observed at 14 DAI within factor A biotypes showed that the herbicide-susceptible RS2 biotype had lower germination than the herbicide-resistant RS1 biotype, with values of 40.1 and 47.2% respectively (Table 1).

For factor B photoperiod, significant differences were observed between the number of hours of light, where 0, 8 and 24 h obtained the highest germination results with 46.3, 50.5 and 52.5% respectively. Treatments with 4, 12, 16 and 20 hours of light had germination equal or lower than 42.0%, significantly lower than in the previous treatments. These results indicated that herbicide-resistant or

Table 1 - Seed germination (%) of *Amaranthus hybridus* L. herbicide-resistant biotype (RS1) and susceptible (RS2) at 14 days after experiment installation (DAI), according to photoperiod.

Germination (%)	
Biotypes	
RS1	47.2 a[1]
RS2	40.1 b.
Photoperiod (hours)	
0 h	46.3 a
4 h	39.0 b
8 h	50.5 a
12 h	35.8 b
16 h	39.3 b
20 h	42.0 b
24 h	52.5 a
CV (%)	21.3

[1]Means followed by the same letter in the column do not differ significantly by the Scott-Knott test ($P \leq 0.05$).

susceptible biotypes of smooth pigweed species do not respond to the presence or absence of light for the initial stimulus of the germination process. Similar findings were reported for *Amaranthus retroflexus* and *A. viridis*, in which light enhanced germination but was not an absolute requirement for seed germination (KHAN et al., 2022). Other results classified the species as positively photoblastic, showing dependence on the incidence of light in the seed germination process (MARTINS, 2020).

Seed germination can occur under different light conditions (Table 1). However, the percentage of normal seedlings from seeds exposed to 8 hours of light showed better percentage of normal plants for both RS1 and RS2 biotypes, with values of 48 and 34%, respectively, showing interaction between the factors (Table 2). In general, increasing the number of hours of light did not improve the percentage of normal plants, with no differences for biotype RS2. In the treatments with 0 or 4 hours of light symptoms of epinasty and twisting were observed in the first root and shoot structures, considered abnormal plants (Figure 1C). Thus, the minimum light regime of 8 h or more has an additive effect for seed germination and survival of seedlings of smooth pigweed, favoring seedling establishment despite not being required to initiate germination (WEI et al., 2023). At latitudes near 30° the daylength exceeds 8 hours during late spring and summer (ABRAHÃO & COSTA, 2018), coinciding with the soybean cultivation period, which has direct practical implications for crop management and smooth pigweed emergence.

Table 2 - Normal plants (%) of *Amaranthus hybridus* L. herbicide-resistant biotype (RS1) and susceptible (RS2), at 14 days after experiment installation (DAI), according to photoperiod.

Photoperiod (hours)	-----Normal plants (%)14 DAI-----	
	RS1	RS2
0 h	0.0 Ad[1]	0,0 Ab
4 h	0.0 Ad	0,0 Ab
8 h	48.0 Aa	34,0 Ba
12 h	38.0 Ab	24,5 Aa
16 h	20.0 Bc	33,5 Aa
20 h	38.0 Ab	27,5 Aa
24 h	50.0 Aa	38,5 Aa
CV (%)	----------19.4----------	

[1]Averages followed by the same lowercase letter in the column do not differ significantly by the Scott-Knott test ($P \leq 0.05$). Averages followed by the same capital letter in the row do not differ significantly using the Scott-Knott test ($P \leq 0.05$).

Air temperature influences germination

No germination was observed at constant temperatures of 10 and 15 °C for either biotype (RS1 and RS2) (Table 3). Therefore, the data could not be fitted to the Weibull model. The same pattern was observed at 40 and 45 °C, except for the biotype RS1 at 40 °C. At 20 °C, T_{50} was 4.90 days for RS1 and 3.78 days for RS2, decreasing to 4.36 and 3.76 days at 25 °C, respectively (Table 3). The shortest germination times were recorded at 30 °C, with T_{50} values of 2.32 days for RS1 and 2.81 days for RS2, indicating this temperature as optimal for germination. At 35 °C, germination occurred only in RS2, with a T_{50} of 6.15 days, while at 40 °C, germination was observed only in RS1, with a T_{50} of 5.98 days, suggesting differences in thermal tolerance between populations (Table 3).

The optimum air temperature (To) for germination of both smooth pigweed biotypes was 28.57 and 28.14 °C and the maximum temperature (Tm) was 44.03 and 42.62 °C, for RS1 and RS2 respectively (Figure 2). Based on these results, it can be determined that air temperature is limited for the germination and establishment of smooth pigweed plants. Low temperatures of less than 15 °C do not allow seeds to germinate, and based on the results for RS1, a temperature of 11.29 °C was estimated as limiting considering the RS2 byotipe (Figure 2).

The temperature range between 28 to 30 °C, should be considered the ideal condition for germination of the species (Table 3, Figure 2) very similar results were observed by CARVALHO & CHRISTOFFOLETI (2007). It should be noted that the Seed Analysis Rule (RAS) (BRASIL, 2009) indicates alternating day/night temperatures of 20/30 °C, corroborating the results obtained in this study with constant temperatures. Further studies using alternating temperature regimes are needed to assess the germination potential of smooth pigweed and to evaluate adaptations or fitness costs associated with herbicide resistance.

Data obtained from the climatological normal for the period between 1991 and 2020 for the city of Santa Maria, Rio Grande do Sul (Brazil), showed that throughout the year the average air temperature is 19.4°C (INMET, 2023), which is not limiting for the germination of the smooth pigweed species. The months between May and September have a minimum temperature of less than 12 °C, which is close to or below the base temperature (Tb) for germination of the species. Maximum temperatures above the limiting level for the establishment of the species (Tm) are not recorded for the region in question. The months between November and

Table 3 - Estimated parameters of the adjusted Weibull function for temperature (ºC) of *Amaranthus hybridus* L. biotypes susceptible (RS2) and resistant (RS1) to herbicides inhibiting acetolactate synthase (ALS) and 5-enolpyruvylchiquimate 3-phosphate synthase (EPSPs).

Temperature (ºC)	----------*a*----------		----------*b*----------		----------*c*----------		----------*d*----------		----------T_{50}---------	
	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2
10	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
15	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
20	0.16	-0.07	65.03	18.00	9.94	8.90	4.93	3.87	4.90	3.78
25	1.13	2.49	1.98	5.46	9.21	5.01	5.26	4.02	4.36	3.76
30	-13.15	-0.01	1.20	18.3	34.86	24.09	3.15	2.87	2.32	2.81
35	Na	1.56	Na	2.06	Na	29.59	Na	7.35	Na	6.15
40	0	Na	8.82	Na	1.51	Na	6.23	Na	5.98	Na
45	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na

Na: parameters not adjusted by the model. T_{50}: time needed to obtain 50% total seed germination.

February stand out, with maximum temperatures close to the optimum temperature (To) for the RS1 and RS2 biotypes (28.57 and 28.14 °C respectively)

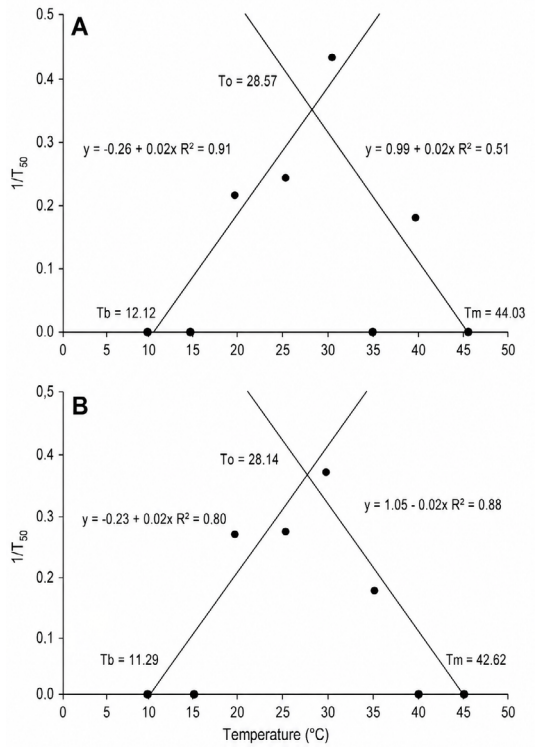


Figure 2 - Linear regressions fitted to the results of $1/T_{50}$ to find the base (Tb), optimum (To) and maximum (Tm) air temperature ranges for the of *Amaranthus hybridus* L. biotypes resistant (RS1) (A) and susceptible (RS2) (B) to herbicides inhibiting acetolactate synthase (ALS) and 5-enolpyruvylchiquimate 3-phosphate synthase (EPSPs).

(Figure 2). These months are considered favorable for the greatest flow of germination and establishment of the smooth pigweed species, coinciding with the main management practices within the soybean crop cycle, around 30 to 60 days after the planting start date, which is released to the region after the month of October (MAPA, 2023). Thus, the species whose temperature requirements are favorable, at a given time when the other environmental factors are adequate, should occupy the niche (RADOSEVICH et al., 1997).

Osmotic potential influences on germination

In the osmotic potential study (Ψo) the germination percentage was influenced according to the level of simulated water stress. There was no interaction between the factors evaluated. No differences were observed between biotypes, ranging from 17.3 to 33.7% for the maximum germination estimated, according to the *c* parameter of the Weibull model (Table 4).

The osmotic potential (Ψo) values of -4, -6 and -8 Bar showed zero or close to zero seed germination, although at -4 Bar, 0.25% germination was observed (data not shown). Due to these results, no data fitting to the Weibull model was obtained for osmotic potentials from -2 to -8 bar (Table 4). These results indicated that when more severe levels of water deficit occur, the seeds are unable to start the germination process. Given the small size of the seeds and their low capacity to store resources such as energy and water, water deficit at these levels becomes the limit for germination of the species.

Most Brazilian soils have a field capacity of between -0.1 Bar for sandy soils and -0.3 Bar for

Table 4 - Estimated parameters of the adjusted Weibull function for osmotic potential levels (Ψo) of *Amaranthus hybridus* L. biotypes susceptible (RS2) and resistant (RS1) to herbicides inhibiting acetolactate synthase (ALS) and 5-enolpyruvylchiquimate 3-phosphate synthase (EPSPs).

Osmotic potential (Ψo)	----------*a*----------		----------*b*----------		----------*c*----------		---------*d*---------		--------T_{50}--------	
	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2
0	-3.30	-0.56	1.92	16.73	26.37	33.7	5.13	3.75	18.11	3.56
-0.25	0	0.12	27.09	18.69	17.15	25.45	3.97	3.84	3.70	3.84
-0.5	-0.02	-0.52	21.75	22.51	18.10	24.95	3.90	3.79	18.90	3.63
-1	0	-0.52	34.65	27.40	18.85	17.30	3.95	3.92	3.70	3.74
-2	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
-4	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
-8	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na

Na: parameters not adjusted by the model. T_{50}: time needed to obtain 50% total seed germination.

clayey soils (MAPA, 2016). At field capacity, soil moisture is generally adequate for seed germination of most species. Germination of smooth pigweed occurred within the range of osmotic potentials evaluated; however, the basal osmotic potential (Ψb) could not be estimated below -2 Bar because the data did not fit the model for lower osmotic potentials for both biotypes (Figure 3). Therefore, although germination decreased with increasing water deficit, seeds remained capable of germinating at osmotic potentials lower than those typically observed at field capacity in most Brazilian soils. Germination in *Amaranthus blitoides* S. Watson and smooth pigweed remains stable only up to approximately −1.0 to −1.2 MPa and declines sharply thereafter, reaching zero at −1.8 MPa (TALAEE et al., 2024). A similar pattern was observed for the RS1 and RS biotypes, in which germination at −1 bar reached maximum estimated values of 18.85% and 17.30%, respectively, as determined by the c parameter of the Weibull model (Table 4).

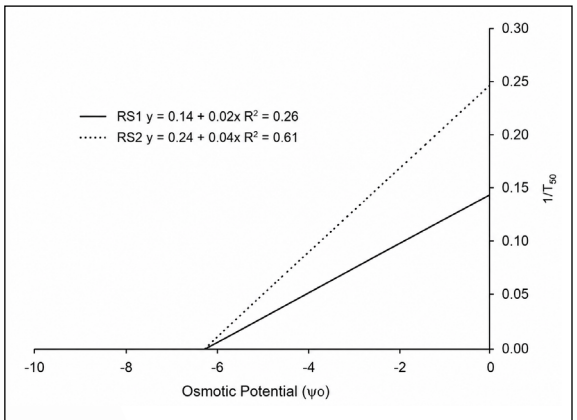


Figure 3 - Linear regressions fitted to the results of $1/T_{50}$ to find the basal osmotic potential levels (Ψb) of *Amaranthus hybridus* L. biotypes susceptible (RS2) (○; dotted line) and resistant (RS1) (●; solid line) to herbicides inhibiting acetolactate synthase (ALS) and 5-enolpyruvylchiquimate 3-phosphate synthase (EPSPs).

Osmotic potential and water availability are major determinants of seed dormancy and germination, although their effects are almost always mediated through interactions with temperature, light, and hormonal signals (KLUPCZYŃSKA E PAWŁOWSKI, 2021). Similar results were found for Niger seeds (*Guizotia abyssinica* (L.) Cass.) where the range between -3 and 4 Bar was observed as the basal osmotic potential (Ψb) (GODIN et al., 2015). A significant reduction in the germination of *Campsis radicans* (L.) Seem (CHACHALIS & REDDY, 2000) and *Solanum sarrachoides* S. (ZHOU et al., 2005) at -2 Bar, for *Senna occidentalis* (L.) Link (NORSWORTHY & OLIVEIRA, 2005) and *Leucaena leucocephala* L. (BORGES et al., 1997) at -4 Bar and *Conyza canadensis* (L.) Cronquist (NANDULA et al., 2006) and *Ipomoea asarifolia* Roem & Schult (DIAS FILHO, 1996) when submitted to osmotic potential (Ψo) of -8 Bar. The results of the smoot pigweed basal osmotic potential (Ψb) study (Table 4, Figure 3) it is important to understand its field occurrence even on water deficit conditions.

Light quality influences germination

The effect of light quality on germination showed a significant interaction between the evaluated factors (Figure 4). There is a difference in the response to the simulation of the different wavelengths. All the treatments simulating the different wavelengths adjusted to Weibull function, except for the blue

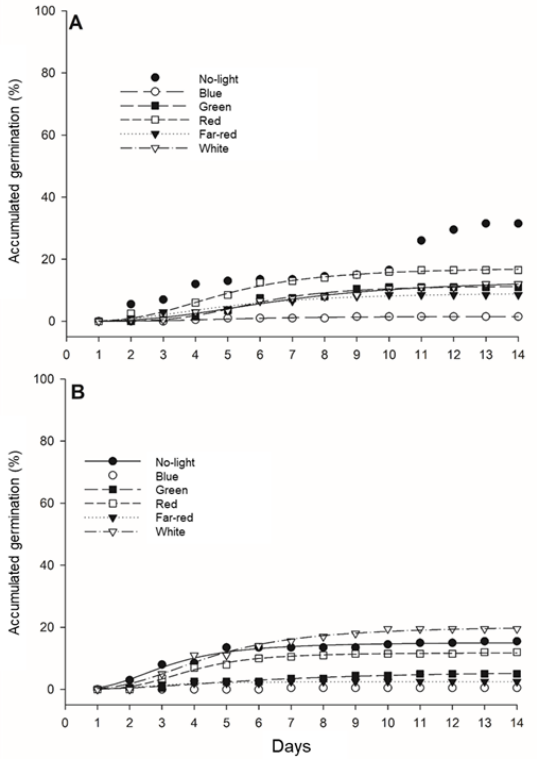


Figure 4 - Cumulative seed germination curves (%) for the levels of light quality for *Amaranthus hybridus* L. biotypes resistant (RS1) (A) and susceptible (RS2) (B) to herbicides inhibiting acetolactate synthase (ALS) and 5-enolpyruvylchiquimate 3-phosphate synthase (EPSPs).

light (Table 5). Blue light in this study simulates a wavelength of 400 nm (MAHMOOD et al., 2016) and had the lowest germination rates during evaluated period (Figure 4).

White light provided a higher T_{50} in the RS1 biotype, while the same behavior was not observed in the RS2 biotype (Table 5). For the T_{50} parameters that were considered significant,

Table 5 - Estimated parameters of the adjusted Weibull function, for the light quality of *Amaranthus hybridus* L. biotypes susceptible (RS2) and resistant (RS1) to herbicides inhibiting acetolactate synthase (ALS) and 5-enolpyruvylchiquimate 3-phosphate synthase (EPSPs).

Light quality	----------*a*----------		----------*b*----------		----------*c*----------		----------*d*----------		---------T_{50}----------	
	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2	RS1	RS2
Absence	Na	-3.27	Na	1.42	Na	14.91	Na	3.34	Na	3.15
Blue	Na	Na	Na	-1.59	Na	Na	Na	Na	Na	Na
Green	-0.19	-0.16	2.93	1.70	11.04	5.29	6.54	6.68	5.78	5.87
Red	-0.07	-1.08	2.23	2.19	16.46	11.60	5.65	4.34	4.88	3.85
Far red	-0.01	-0.15	2.05	1.85	8.63	2.48	5.58	3.38	4.85	2.87
White	-0.29	-2.36	2.11	1.70	12.20	19.70	7.46	4.97	6.72	4.44

Na: parameters not adjusted by the model. T_{50}: time needed to obtain 50% total seed germination.

the lowest value was observed for the far-red light condition, indicating that in this condition there was an activation of the phytochromes due to the longer wavelength that reached the seeds. Phytochromes are activated in wavelength ranges close to the red filter, between 620-750 nm, and are important for triggering the biosynthesis of gibberellin, which is fundamental for inducing the germination process of seeds (JIA et al., 2018). These results have implications for confirming the ability to seed germination with little or no light. In this case, management practices such as crop sowing in no-tillage systems with straw on the soil may not prevent the germination of the species. Further studies should be conducted to assess smooth pigweed emergence conditions under straw cover and with low light intensity reaching the soil.

CONCLUSION

The optimum temperatures for germination of the smooth pigweed is 28.57 and 28.14 °C for the herbicide-resistant (RS1) and herbicide-susceptible (RS2) biotypes respectively. Germination of the species can occur in temperature conditions between 11.12 and 44.03 (RS1) or 42.62 (RS2), these being the estimated basal and maximum temperatures.

Smooth pigweed has neutral photoblastic seeds, with light being an additive but not limiting factor. This condition is evidenced by the effect of light quality on germination, with the lowest T_{50} values under far-red light conditions for both biotypes. Severe levels of water deficit, when the osmotic potential (Ψo) is below -2 Bar, prevents smooth pigweed seed germination due to limited water uptake. The ability to germinate under moderate water stress indicates an ecological strategy that allows the species to establish under variable soil moisture conditions rather than tolerance to extreme drought.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

Article conceptualization and methodology development: ST and AU; data collection and curation: ST, RM, AC, SP and JB; data analysis: ST, SP and RN; data interpretation: ST, AU, RM and RN; acquisition of funding and resources: ST and AU; obtaining seeds: ST, CO and AU; project administration: ST and AU; supervision: AU; writing of the original version of the manuscript: ST.; writing, revising and editing: ST, AU, AS, CO, RM, RN and SP. All the authors have read and agreed with the published version of the manuscript.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence (AI) tools were used exclusively to assist with language editing, translation, and improvement of the manuscript's readability. All AI-generated suggestions were critically reviewed, edited, and approved by the authors, who assume full responsibility for the accuracy and integrity of the scientific content.

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