Research Article

Water restriction, salinity and depth influence the germination and emergence of sourgrass

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HIGHLIGHTS

- Osmotic potential and salinity can restrict sourgrass seed germination.
- Salinity can improve the occurrence of abnormal sourgrass seedlings.
- Sourgrass seedlings can emerge from relatively deep soil depth despite its small size.

ABSTRACT

Background: Substantial losses to crops can occur due to the presence of sourgrass in agroecosystems, which is promoted by its seed dispersal ability. Environmental factors can affect sourgrass germination and emergence.

Objective: The objective of this study was to determine the effects of water restriction, salinity, and depth of sowing on the sourgrass germination and emergence processes.

Methods: Experiments using sourgrass seeds were conducted under controlled conditions simulating osmotic and saline stress and seeding depths. The data collected was submitted to analysis of variance and adjusted to models by regression analysis.

Results: Smaller osmotic potentials reduced germination, normal seedlings, and increased quiescence, with no germination occurring from -0.80 MPa. Salinity caused a decrease in germination from 20 mM NaCl, influencing the percentage of normal and abnormal seedlings and quiescent seeds. Seed depth influenced the emergence of seedlings, with the maximum percentage of emergence (83%) in the seeds placed on the substrate surface. The maximum depth from which sourgrass seedlings emerged was 7.6 cm.

Conclusions: The results lead to the conclusion that the sourgrass germination process tolerates low osmotic potentials and salinity levels. Despite being small sized seeds, they can emerge from high depths. These characteristics justify its occurrence in several regions and agroecosystems, especially in perennial and no-till crops lacking soil cover.

1 INTRODUCTION

The perennial monocotyledonous popularly known as sourgrass (*Digitaria insularis* (L.) Nees ex Ekman) has C4 photosynthetic metabolism. Corresponds to one of the 391 species of the *Digitaria* genus that occur globally (Clayton et al., 2002). Rhizomatous and clumping, the plant is native to the tropical and subtropical regions of America, extending from Argentina to the southern United States. It has

striated stems and long internodes, from 50 to 150 cm high, leaves with long and hairy sheath and membranous ligule (Lorenzi, 2008).

Sourgrass infestations usually take place in pastures, crops, orchards, roadsides, and empty pieces of land. This species has become a major weed in some no-tillage systems due to the lack of soil movement that allows its perennialization, consequently, hindering chemical control. Its dissemination and survival are increased by the occurrence of biotypes that are resistant to EPSPs and ACCase herbicides in Brazil, Argentina and Paraguay (Heap, 2018).

Experiments conducted to determine the negative impact of sourgrass on agricultural crops are still scarce in the literature, but it is known that soybeans may suffer 44% yield reduction (Gazziero et al., 2012). It also causes problems in perennial crops, including citrus, coffee and pasture, especially after generating rhizomes that accumulate reserves allowing rapid regrowth after management (Machado et al., 2008).

The high invasive potential of sourgrass can be recognized by its ability to propagate both from rhizomes and seeds. While rhizome propagation is more common in an already infested area, the invasion of new areas depends on seed dispersion. The plants emit inflorescences during hot seasons and their panicles have a high number of hairy seeds with high germinative power, being disseminated with the help of the wind (Kissmann and Groth, 1999).

With high viability and low dormancy, sourgrass seeds can reach germination percentages greater than 90% in the absence of photoperiod and with alternating temperatures of 20/35 °C (Oreja et al., 2017). The reasons that determine the effects of alternate temperature are not fully known, but it is assumed that this variation in thermal amplitude may create a change in the hormonal balance between germination promoters and inhibitors (Footitt et al., 2011; Huarte and Benech-Arnold, 2010). When in constant temperature of 25 or alternating 20/30 °C, sourgrass germination is favored by the presence of light (Mondo et al., 2010).

The germination speed index of *D. insularis* seeds is greater than 20 under optimal conditions. This value is sometimes greater than that observed in *D. bicornis*, *D. ciliaris* and *D. horizontalis* seeds (Mondo et al., 2010; Pyon et al., 1977). This characteristic of sourgrass can counterbalance its slow initial development up to the 45th day after emergence (Machado et al., 2006), because early seedling emergence provides competitive advantage over other weeds or cultivated species.

Although there are studies on sourgrass ecology, physiology and botany, information on its germination under water and saline stress is scarce, as well as the effect of seed sowing depth on seedling emergence. This work aimed to evaluate sourgrass germination and emergence under different osmotic potentials, saline stresses, and sowing depths.

2 MATERIALS AND METHODS

The experiments were conducted at the Universidade Federal da Fronteira Sul, in the Weed Science, Plant Physiology, and Germination and Plant Growth laboratories.

The experimental design used in all experiments was completely randomized with four replications. The experiments were conducted in growth chambers with controlled temperature and photoperiod, model BF2 CGFP 295 from Biofoco. Alternated temperature of 20/35 °C and light cycles every 12 hours were used.

2.1 Seed collection and preparation

When the seeds reached dispersal condition, the panicles from 100 sourgrass individuals were collected randomly in March 2018 in an infested field of 9.6 hectares in the municipality of Catanduvas (Paraná, Brazil). The collected panicles were dried in the shade for a period of 10 days, and then the seeds were separated, homogenized, and kept at room temperature (~ 22 °C) until use.

2.2 Water restriction experiment on sourgrass seeds

The sourgrass germination process was evaluated under different osmotic potentials. The experimental units consisted of Petri dishes with 50 seeds, arranged evenly and wrapped with plastic film to avoid moisture loss. The dishes were prepared with two layers of blotting paper and moistened with PEG 6000 (polyethylene glycol) solutions in the 2.5:1 ratio (solution volume/paper mass), in concentrations equivalent to the osmotic potentials (ψs) of 0.00, -0.05, -0.10, -0.20, -0.40, -0.80 and -1.20 MPa and incubated. The calculation of the solution osmotic potential was obtained by adapting Michel and Kaufmann (1973) equation (Equation 1).

$$\Psi s = 0.1 * [-(1.18 * 10^{-2}) * C - (1.18 * 10^{-4}) * C^2 + (2.67 * 10^{-4}) * C * T + (8.39 * 10^{-7}) * C^{-2} * T]$$

(eq. 1)

where: *C* is the concentration in grams of polyethylene glycol per kilogram of distilled water and *T* is the temperature in ℃.

The sourgrass germination was evaluated daily for 10 days, when germination stabilized. The seeds whose root protrusion was visible were considered germinated. At the end of the tenth day, normal and abnormal seedlings were counted, as well as dead and quiescent seeds.

The germination speed was determined by the germination speed coefficient (GSC) proposed by Nichols and Heydecker (1968) (Equation 2).

$$\text{GSC (\%)} = \left(\frac{\sum ni}{\sum ni*xi}\right) * 100 \qquad \text{(eq. 2)}$$

where: *ni* is the number of seeds germinated on the i-th day, and *xi* is the number of days counted from sowing to the day of evaluation.

The germination or uncertainty synchronization index (Ē) was determined using Labouriau (1983) equation (Equation 3).

$$\bar{\text{E}} = -\sum_{i=1}^{k} fi \, log_2 \, fi \qquad \text{(eq. 3)}$$

where: *fi* is the relative frequency of germination, *log2* is the base 2 logarithm and *k* is the last day of evaluation.

The germination synchronization index in the different osmotic potentials was adjusted, based on regression analysis, in a quadratic polynomial model. The point for the maximum value of the synchronization index (X) was determined by interpolation (Equation 4).

$$\text{X} = \frac{-b1}{2*b2} \qquad \text{(eq. 4)}$$

where: *b1* and *b2* are the parameters of the quadratic polynomial model.

Normal seedlings were those that presented well-developed structures, with conditions to become an adult plant. Symptoms of poor development of the shoots and/or root system characterized abnormal seedlings.

Dead seeds were understood as those that released purulent-looking secretion after being pressed. Normal looking ungerminated seeds were considered quiescent, which remained firm when pressed. The evaluations of normal seedlings, and dead and quiescent seeds were performed 10 days after sowing.

2.3 Salinity experiment on sourgrass seeds

The salinity experiment followed the same procedures already described. Salt stress was simulated with solutions containing NaCl in concentrations of 0, 10, 20, 40, 80, 120 and 200 mM in distilled water. The daily count of germinated seeds was evaluated for 10 days, when normal and abnormal seedlings, and dead and quiescent seeds were counted.

The germination synchronization index in the different salinities was adjusted, based on regression analysis, in a cubic polynomial model. The maximum value point of the synchronization index (X) was determined by interpolation (Equation 5).

$$\text{X} = \frac{(-2*b2 \pm \sqrt{4*b2^2 - 12*b1*b3})}{6*b3} \qquad \text{(eq. 5)}$$

where: *b1*, *b2* and *b3* are the parameters of the cubic polynomial model.

2.4 Sourgrass emergence from different sowing depths experiment

The sourgrass emergence from 0; 2; 4; 6 and 8 cm from the substrate surface was evaluated. The substrate consisted of soil (distrophic Red Latosol) and sand in a 2: 1 ratio (v/v). Soil and sand were sieved (2 mm) and subsequently autoclaved for 15 minutes. The substrate was allocated in 300 ml plastic pots, moistened until reaching 80% of the field capacity and 25 seeds per pot were sown. The total of emerged plants was performed on the sixteenth day after sowing, when emergence stabilized. Visible leaflets on the substrate surface characterized emerged seedlings.

2.5 Statistical analysis

The data obtained from the variables of all experiments were submitted to analysis of normality and variance with subsequent regression analysis using the Genes software (Cruz, 2013).

3 RESULTS AND DISCUSSION

3.1 Water restriction on sourgrass seeds

The osmotic potential influenced sourgrass germination, with the maximum germination percentage (82%) observed in the absence of water restriction (0 Mpa) (Figure 1). Germination decreased as water availability was reduced, with no germination from -0.80 MPa. Water availability is a limiting factor in sourgrass germination, as well as for most plant species. This is due to the absorption and hydration processes of the seed tissues, as they are

The dots represent the means of the treatments and the bar the corresponding standard error.

Figure 1 - Relationship between the sourgrass accumulated germination (%) and time (days after sowing).

0.00 MPa | $Y = -2.057 + (82.07 + 2.057)/1 + \exp [(3.122 - X)/0.3808] | R^2 = 1.00$ |
-0.05 MPa | $Y = -2.238 + (73.15 + 2.238)/1 + \exp [(3.417 - X)/0.5082] | R^2 = 1.00$ |
-0.10 MPa | $Y = -5.314 + (68.51 + 5.314)/1 + \exp [(3.946 - X)/0.8813] | R^2 = 0.99$ |
-0.20 MPa | $Y = -1.840 + (46.30 + 1.840)/1 + \exp [(4.587 - X)/0.8256] | R^2 = 0.99$ |
-0.40 MPa | $Y = -0.174 + (26.98 + 0.174)/1 + \exp [(6.910 - X)/1.0290] | R^2 = 1.00$ |

$$Y = 25.46 + 31.51*X$$
$$R^2 = 0.99$$

The dashed line identified by D_{50} corresponds to the osmotic potential associated with half of the maximum germination speed coefficient.

Figure 2 - Relationship between the germination speed coefficient of sourgrass seeds and the osmotic potential (MPa) of PEG 6000 solution (polyethylene glycol).
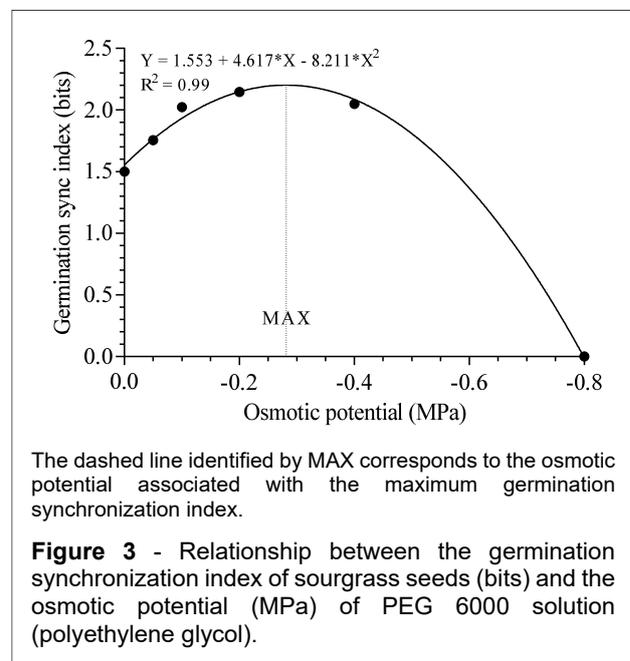
fundamental for the occurrence of an ordered sequence of metabolic events that result in embryonic development and seedling growth (Marcos Filho, 2015).

The increase in water restriction decreased the germination speed coefficient (GSC) of sourgrass seeds, whose maximum value (25) was reduced by 50% with -0.40 MPa (Figure 2). The reduction in GSC with the decrease in the osmotic potential of the seeds substrate is the rule for several other plant species. This is primarily due to the reduction in the water penetration speed through seed tissues to reach the embryo, causing a reduction in cell turgor and affecting cell expansion and division (Marcos Filho, 2015; Taiz et al., 2017). Plant cells expand ten to ten thousand times in volume before reaching maturity, depending on water to provide the necessary turgor pressure for the extension of the cell wall (Taiz et al., 2017).
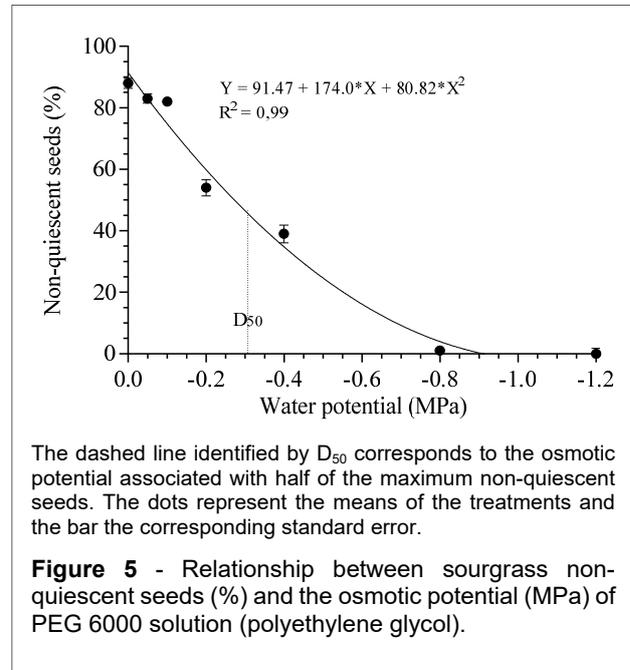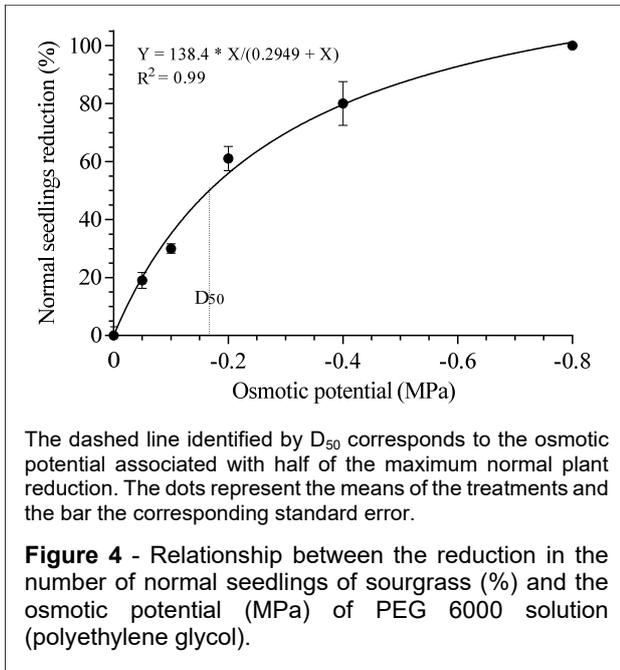
The reduction in GSC is usually seen when water is retained by the substrate with tension greater than -0.2 MPa (Meneses et al., 2011; Rossetto et al., 1997). However, there are species, such as wheat and carrots, that are more tolerant of water restriction, reducing GSC only from -0.8 MPa downwards (Armondes et al., 2013). For sourgrass seeds, it was possible to verify a linear relationship between the osmotic potential and the GSC, in which the reduction of every 0.1 MPa in water potential resulted in a reduction of ~3% in the GSC.

The germination synchronization index (GSI) was also influenced by the osmotic potential, with a maximum value of 2.2 bits in -0.28 MPa (Figure 3). The higher values of the synchronization index indicate

$$Y = 1.553 + 4.617*X - 8.211*X^2$$
$$R^2 = 0.99$$

The dashed line identified by MAX corresponds to the osmotic potential associated with the maximum germination synchronization index.

Figure 3 - Relationship between the germination synchronization index of sourgrass seeds (bits) and the osmotic potential (MPa) of PEG 6000 solution (polyethylene glycol).

less synchrony in seed germination, suggesting that in the osmotic potential of -0.28 MPa the germination process was triggered a greater number of times than in the other water potentials. This irregularity in germination may favour sourgrass survival, since the seedlings will tend to emerge at different times in natural conditions, allowing part of the progeny to escape from adverse climatic conditions (Larcher, 2000) or management in agroecosystems.

The osmotic potential had a significant effect on germination, with the highest percentage of normal seedlings (79%) observed at 0 MPa (Figure 4). There was a proportional relationship between the reduction in normal seedlings and the reduction in osmotic potential. While -0.16 MPa was the value for 50% reduction (D50) in the maximum normal

$Y = 138.4 * X/(0.2949 + X)$
$R^2 = 0.99$

The dashed line identified by D_{50} corresponds to the osmotic potential associated with half of the maximum normal plant reduction. The dots represent the means of the treatments and the bar the corresponding standard error.

Figure 4 - Relationship between the reduction in the number of normal seedlings of sourgrass (%) and the osmotic potential (MPa) of PEG 6000 solution (polyethylene glycol).

$Y = 91.47 + 174.0 * X + 80.82 * X^2$
$R^2 = 0.99$

The dashed line identified by D_{50} corresponds to the osmotic potential associated with half of the maximum non-quiescent seeds. The dots represent the means of the treatments and the bar the corresponding standard error.

Figure 5 - Relationship between sourgrass non-quiescent seeds (%) and the osmotic potential (MPa) of PEG 6000 solution (polyethylene glycol).

seedlings, -0.71 MPa reduced 100%. Decrease in the percentage of normal seedlings with the reduction in osmotic potential is also common for cultivated Poaceae species (Campos and Assunção, 1990).

The reduction in germination percentage is generally affected at higher osmotic potential stresses, between -0.4 and -0.6 MPa, when compared to what is required for GSC reduction (Marcos Filho, 2015). Root protrusion, in most species, is primarily the result of cell expansion, which is dependent on the availability and absorption of water by the cell to generate the turgor pressure that potentiates the process (Nonogaki et al., 2010). Thus, it is possible that the reduction in the osmotic potential of the solution, may have interfered in the seeds cell expansion and, in a second moment, in the cell division, causing abnormal development. Water is essential for several metabolic processes in the plant, including the formation of basic cell structures on which the normal constitution of plant tissues depends, thus justifying the increase in the percentage of abnormal seedlings with the increase in water restriction.

Seed quiescence was directly affected by the osmotic potential reduction (Figure 5). The quiescence increased in a quadratic way from osmotic potentials of 0.0 and -0.80 MPa, sustaining the amount of quiescent seeds until -1.2 MPa. The maximum percentage of quiescence (97%) was observed in -0.80 MPa. With the data normalization, the relationship between non-quiescent seeds and the osmotic potential could be observed.
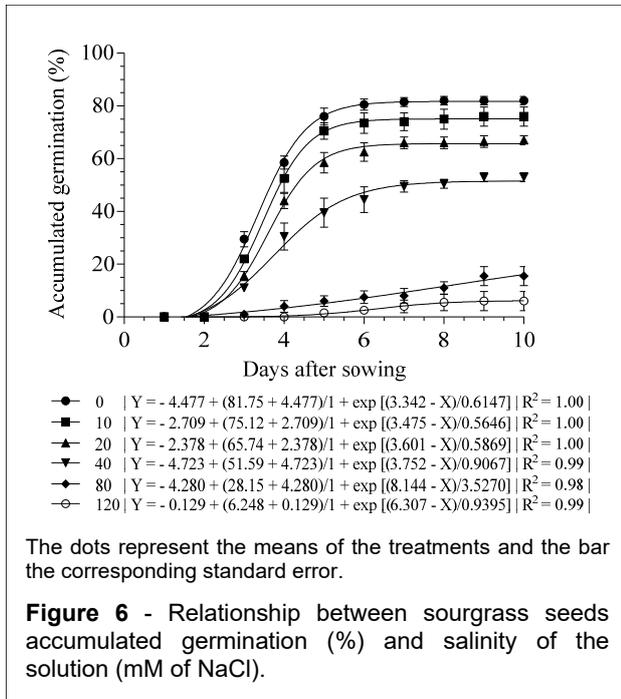
The maximum percentage of non-quiescent seeds was reduced by 50% at -0.31 MPa. Differential

sensitivity to the availability of water (osmotic potential) necessary for germination to occur is common between individuals of the same population. Some seeds of the population can germinate in more negative potentials while others cannot. This differential sensitivity between individuals from the same population is a great ecological strategy to overcome drought, as it allows intersperse germination over time (Bewley et al., 2013). In addition, sites with differential water availability in the soil can also generate differentiation in the percentage of quiescent seeds and consequently multiply emergence flows, making these weeds difficult to manage in agroecosystems.

The seeds are tolerant to desiccation until the radicle protrudes. After that, they become more sensitive, easily dying from water restriction (Nonogaki et al., 2010). For this reason, quiescent seeds that can interrupt their germination process before protrusion, may indicate an important survival characteristic to water stress. Although desiccation resistance has not been evaluated in this study, it has already been verified in another Poaceae, the South African lovegrass (*Eragrostis plana*) (Bittencourt et al., 2017).

3.2 Salinity in sourgrass seeds

Salinity influenced the germination of sourgrass seeds. An inversely proportional relationship was observed between saline concentration and seed germination. The highest germination (82%) occurred at 0 mM NaCl (Figure 6). However, no germination was observed at 200 mM (not shown in Figure 6). The reduction in germination can be explained by the

The dots represent the means of the treatments and the bar the corresponding standard error.

Figure 6 - Relationship between sourgrass seeds accumulated germination (%) and salinity of the solution (mM of NaCl).

The dashed line identified by D_{50} corresponds to salinity associated with half of the maximum germination speed coefficient.

Figure 7 - Relationship between the sourgrass seeds germination speed coefficient and the salinity of the solution (mM of NaCl).

The dashed line identified by MAX corresponds to the salinity associated with the highest seed germination synchronization index.

Figure 8 - Relationship between sourgrass seeds germination synchronization index (bits) and salinity of the solution (mM of NaCl).

osmotic and toxic effect of NaCl on the seeds, reducing water absorption and increasing the entrance of toxic ions (Lima et al., 2005). In addition to the reduction in germination, salinity can negatively influence the germination speed (Jones, 1986).

The sourgrass GSC was reduced with the increase in the solution salinity. Maximum GSC (~24) was registered with 0 mM NaCl, and suffered a 50% reduction by 122.8 mM NaCl (Figure 7). The availability of water affects both the speed and the percentage of germination, with a reduction in the speed of germination preceded by a drop in germination. In a more negative osmotic potential, water penetrates the tissues of the seeds slowly, decreasing the turgor pressure of the cells of the embryo and consequently the cell expansion, responsible for root protrusion (Bewley et al., 2013).
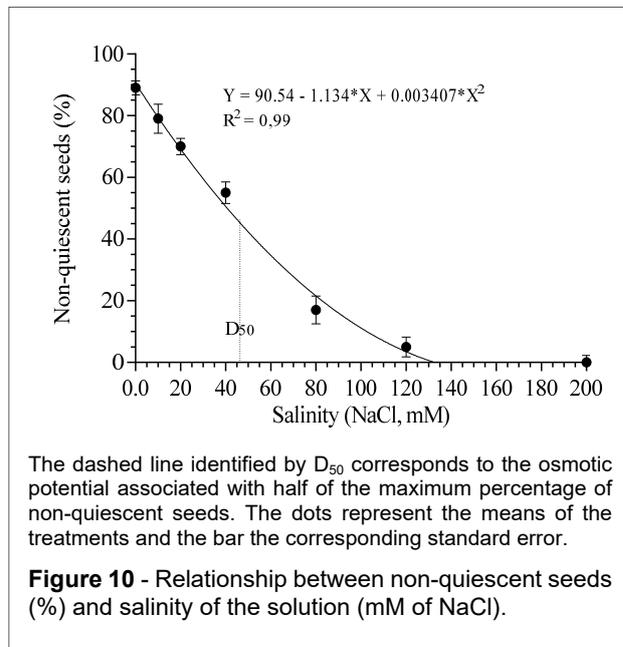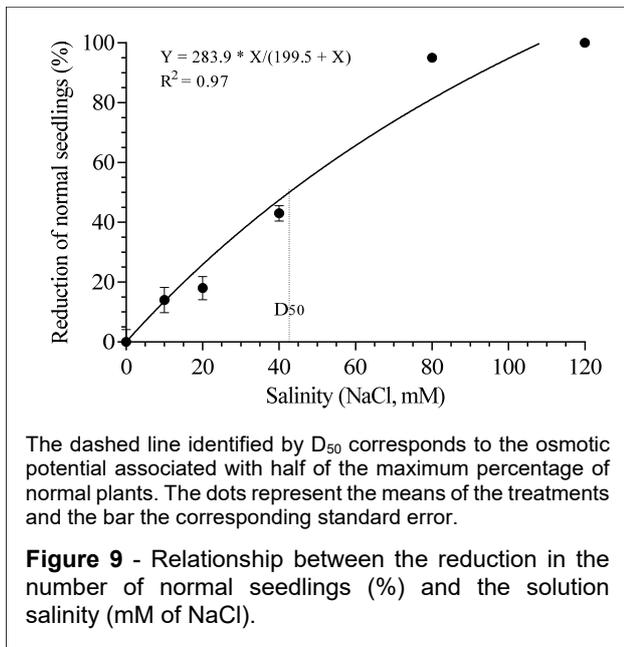
The Germination Synchronization Index was influenced by salinity, reaching the maximum value (2.1 bits) with 38.4 mM NaCl (Figure 8). The maximum GSI value in the salinity test is equivalent to that observed in the water restriction test (2.2 bits), confirming that different environmental factors may influence the organization of the germination process (Lopes and Franke, 2011), and consequently make the seeds germinate at different times. Thus, this response could decrease the plants' exposure to salinity concentrations that are harmful.

Salinity showed an inversely proportional relationship to the generation of normal seedlings. That is, there was a decrease in normal seedlings with
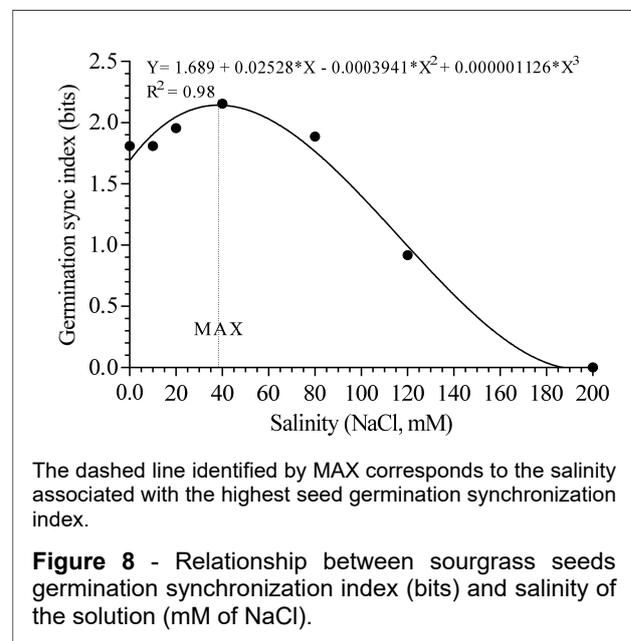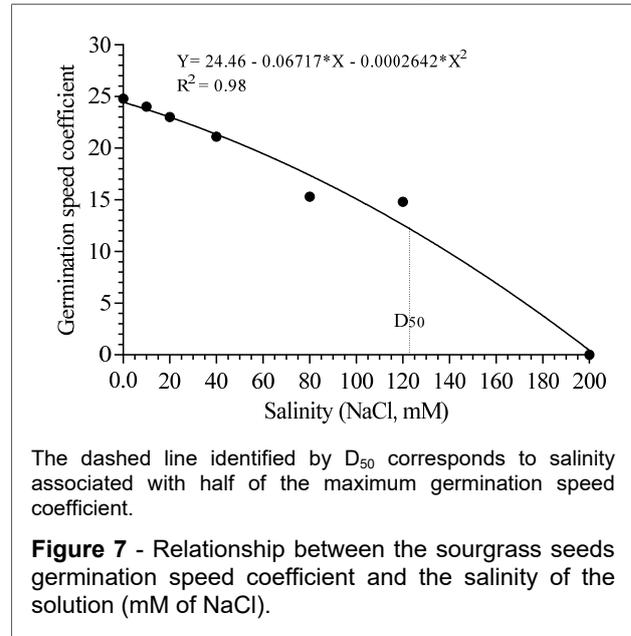
the increase in saline concentration. The formation of normal seedlings followed a trend like the water restriction experiment, simulated with PEG 6000.

The percentage of normal sourgrass seedlings was reduced with the increase in salinity, justified by the increase in the number of abnormal seedlings or/and quiescent seeds. The salinity responsible for the 50% reduction in normal seedlings was 42.6 mM NaCl (Figure 9). The reduction in the percentage of normal seedlings with increased salinity is expected, as after a certain concentration of the salts in the solution there are osmotic imbalances and cell intoxication. These can compromise the germination process, vegetative and reproductive growth of the plants and the quality of produced seeds (Aydinşakir et al., 2015).

The dashed line identified by D_{50} corresponds to the osmotic potential associated with half of the maximum percentage of normal plants. The dots represent the means of the treatments and the bar the corresponding standard error.

Figure 9 - Relationship between the reduction in the number of normal seedlings (%) and the solution salinity (mM of NaCl).

The dashed line identified by D_{50} corresponds to the osmotic potential associated with half of the maximum percentage of non-quiescent seeds. The dots represent the means of the treatments and the bar the corresponding standard error.

Figure 10 - Relationship between non-quiescent seeds (%) and salinity of the solution (mM of NaCl).

Salinity also influenced the formation of abnormal seedlings with the higher occurrence of abnormal seedlings (17.5%) at 10 mM (Figure 9). From that point on, a gradual reduction was registered as the salinity increased, until it reached 0% with 200 mM NaCl. This effect can be due to the absence of seed germination (data of abnormal seedlings not shown). The tolerance to salts varies according to the plant development stage, and the response to salinity is strictly linked to the time of exposure. Therefore, further studies with longer exposure time may complement these observations, allowing a better understanding of the relationship between sourgrass seedling development and salinity.

The increase in salinity gradually promoted seed quiescence. A rise in seed quiescence was observed as the NaCl concentration was increased, with 46.38 mM responsible for 50% reduction in the maximum value of readily germinating seeds (Figure 10). The increase in quiescence in higher concentrations can be explained by the reduction in the osmotic potential caused by NaCl (LIMA et al., 2005).

The reduction in sourgrass germination by a relatively low saline concentration (46.38 mM NaCl), suggests that this species is not adapted to salinity (glycophyte). The species adapted to salinity (halophyte) normally have high germination rate up to 100 mM salt concentrations (Ungar, 1991). This study does not allow to conclude if the inhibition on the sourgrass germination was due to the osmotic or cytotoxic effect from Na^+ and Cl^-. Because these ions are beneficial to plants at low concentrations,
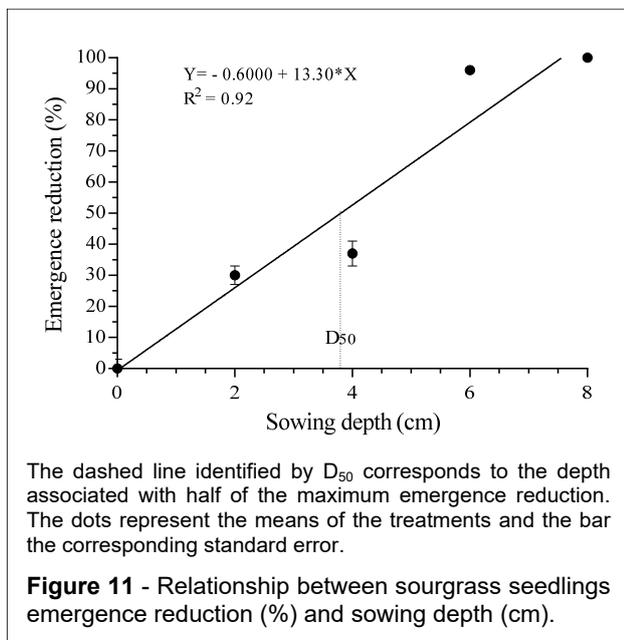
while at high concentrations they can cause protein denaturation and membrane destabilization (Taiz et al., 2017).

If the inhibition of seed germination occurred mainly due to the osmotic effect of salt, as evidenced when comparing these results with those from the tests with PEG 6000 (Figure 1), this can be considered a survival strategy, since the seeds become intolerant to desiccation after protrusion of the radicle (Nonogaki et al., 2010). Therefore, future studies can investigate the sourgrass seeds tolerance to the toxic effect of NaCl, as the percentages of abnormal seedlings were comparable to the tests with PEG 6000, which has no toxic effect (Michel and Kaufmann, 1973).

3.3 Sourgrass emergence from different depths

The depth of sowing significantly influenced the sourgrass emergence. There was a linear reduction in seedling emergence with increasing sowing depth, with 3.8 cm being the depth responsible for reducing the maximum seedling emergence by 50% (Figure 11). The largest number of emerged plants was 83%, observed in seedlings on the substrate surface (0 cm). It is assumed that the emergence did not occur from 7.6 cm, mainly due to two factors: reduced temperature or thermal amplitude and the limited amount of seed reserves. The emergence of seedlings up to 7.6 cm deep confirms the insensitivity of the sourgrass seed phytochrome to the red and distant red light ratio for the occurrence of germination (Mondo et al., 2010).

The initial development stages correspond to the period in which plants, including the sourgrass, are

The dashed line identified by D_{50} corresponds to the depth associated with half of the maximum emergence reduction. The dots represent the means of the treatments and the bar the corresponding standard error.

Figure 11 - Relationship between sourgrass seedlings emergence reduction (%) and sowing depth (cm).

most susceptible to the weed management programs adopted by farmers. Even though it is a neutral species in relation to photoblasty, it is possible that higher volumes of phytomass produced by cover crops in no-tillage systems may negatively influence the emergence process. This can be expected as soil cover reduces the soil temperature and its fluctuation and can also form a physical barrier to inhibit plant establishment. It is also suggested that further research be developed to elucidate which species of cover crops are most efficient in controlling sourgrass emergence and initial development.

4 CONCLUSIONS

Our results led to the conclusion that negative osmotic potentials and salinity reduce germination, vigor and synchrony of sourgrass germination. The sourgrass seeds show glycophyte species behavior, with a 50% decrease in the maximum value of germinable seeds in the 46.38 mM NaCl concentration Sourgrass seeds can emerge from up to 7.6 cm depth in the soil.

5 CONTRIBUTIONS

JZ, HVHB, and MMT:conceived and planned the experiments. JZ: carried out the experiments. JZ, HVHB, LTSB, and ACPPF: contributed to the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

6 ACKNOWLEDGMENTS

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