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Recovery of *Bradyrhizobium* cells and effects on the physiological quality of soybean seeds sown in dry soil

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ABSTRACT: Farmers sometimes sow soybean (*Glycine max* (L.) Merrill) in dry soil in the expectation of rain in the short time. However, the forecast may not confirm, letting the inoculated seeds in the dry soil indefinitely. We assessed the survival of inoculated *Bradyrhizobium* and physiological quality of soybean seeds sown in dry soil. In the first experiment, irrigation was applied with 2 h, 1, 4, 11, 18, or 21 days after sowing; in the second experiment, sowing was carried out 2 h, 1, 5, 12, 14, or 20 days before irrigation. Each time represented a treatment in a completely randomized design. *Bradyrhizobium* cells dropped from ~8-9 × 10^4 colony forming units per seed soon after inoculation to -60% at 2 h after sowing in dry soil, and decreased to close to zero with time in both experiments. Although there was no effect on germination (59% and 81% in the first and second experiments, respectively), the exposure to dry soil reduced the emergence speed index from 19.5 (2 h) to 12.0 (21 days) in the first experiment and from 37.8 (2 h) to 13.8 (21 days) in the second. In the first experiment, the number of abnormal seedlings increased from 7% (2 h) to 24% (21 days); in the second, cotyledons showed cracks, which increased from 1% (2 h) to ~50% (≥ 5 days). Sowing in dry soil negatively affects not only the inoculated *Bradyrhizobium*, but also the physiological quality of soybean seeds.

Index terms: drought, emergence speed index, inoculation, seed physiological quality.

RESUMO: Em alguns casos os agricultores semeiam a soja (*Glycine max* (L.) Merrill) no solo seco, na espera de ocorrência de chuvas no curto prazo. Entretanto, a previsão pode não se confirmar, fazendo com que as sementes inoculadas permaneçam no solo seco indefinidamente. Avaliou-se a sobrevivência de *Bradyrhizobium* e o efeito sobre a qualidade fisiológica de sementes de soja inoculadas e semeadas em solo seco. No primeiro experimento, a irrigação foi aplicada com 2 h, 1, 4, 11, 18 ou 21 dias após a semeadura; no segundo experimento, a semeadura ocorreu 2 h, 1, 5, 12, 14 ou 20 dias antes da irrigação. Cada tempo representou um tratamento em delineamento inteiramente casualizado. A recuperação de *Bradyrhizobium* caiu de ~8-9 × 10^4 unidades formadoras de colônias por semente logo após a inoculação para -60% 2 h após a semeadura em solo seco, e tendeu a zero com o tempo, em ambos experimentos. Apesar de não afetar a germinação (59% e 81% nos no primeiro e no segundo experimento, respectivamente), a exposição ao solo seco diminuiu o índice de velocidade de emergência de 19,5 (2 h) para 12,0 (21 dias) no primeiro e de 37,8 (2 h) para 13,8 (21 dias) no segundo. No primeiro experimento, o número de plântulas anormais aumentou de 7% (2 h) para 24% (21 dias); no segundo, os cotilédones apresentaram trincas, que aumentaram de 1% (2 h) para ~ 50% (≥ 5 dias). A semeadura em solo seco prejudica não apenas o *Bradyrhizobium* inoculado, mas também a qualidade fisiológica das sementes de soja.

Termos para indexação: seca, índice de velocidade de emergência, qualidade, inoculação, fisiológica de sementes.

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INTRODUCTION

The high protein content in soybean (*Glycine max* (L.) Merrill.) grains results in high nitrogen (N) requirement, about 80 kg for each ton of grains, of which 65 kg are exported and 15 kg are used in the structural development of the plant (Seixas et al., 2020). A yield of 3,500 kg.ha^{-1} requires 280 kg.ha^{-1} of N, most of which can be supplied by biological nitrogen fixation (BNF), performed by bacteria of the genus *Bradyrhizobium* in symbiosis with plant roots.

Adequate inoculation of soybean seeds provides diazotrophic bacteria in the quantity and quality necessary for early establishment of the symbiosis (Hungria et al., 2017), since the population established in the soil may be physiologically limited by several environmental factors, besides not necessarily being the most efficient in performing BNF (Hungria and Mendes, 2015). Inoculation with elite strains increases the number of bacteria that are physiologically active and efficient in performing BNF, stimulating the rapid formation and occupation of nodules, which results in more N fixed to the plant. Soybean inoculation must provide at least 1.2 million cells per seed, from which 80,000 to 100,000 must be recovered at the time of sowing (Hungria et al., 2017). This is a number considered sufficient to promote adequate soybean root nodulation in areas with an established *Bradyrhizobium* population.

Before increasingly intensive production systems, with two or three crops per agricultural cycle, farmers have advanced the soybean sowing to establish the next crop as early as possible, such as second-season maize (*Zea mays* L.) or cotton (*Gossypium hirsutum* L.), aiming to take advantage of soil moisture at the end of the rainy season (Ferrari et al., 2015). In several soybean producing regions of Brazil, the crop has been planted at the end of the sanitary break, a period in which the rainfall cycle is not yet regularized in most production areas. Many farmers in non-irrigated areas perform the sowing operation without sufficient soil moisture for germination, which is popularly known as dry sowing, expecting that rains will occur in the hours or days following sowing to induce germination. However, this period can vary from a few hours to several days, which may compromise not only the physiological quality of the seeds, but also the survival of *Bradyrhizobium* cells inoculated in them, which remain exposed to a harsh environment that can negatively influence BNF and the crop yield potential.

Another factor that aggravates the survival of inoculated bacteria is the treatment of seeds with insecticides, fungicides, nutrients and several other products, including biological products, intensifying the negative effects of hostile soil conditions on the survival of *Bradyrhizobium* (Santos et al., 2019; Rodrigues et al., 2020). The combination of high temperatures, commonly found in dry soil, and the presence of substances used in the treatment of seeds and in contact with the cells of inoculated bacteria, further hampers their survival at a ratio directly proportional to the exposure time (Hungria and Nogueira, 2019). In addition, the permanence of seeds in contact with chemicals for long periods, especially under high temperatures, compromises their physiological quality (Brzezinski et al., 2015; Abati et al., 2020).

The aim of this study was to evaluate the recovery of viable *Bradyrhizobium* cells inoculated in soybean seeds, sown and maintained for different periods of exposure to dry soil, as well as their effect on seed germination and initial seedling development.

MATERIAL AND METHODS

Two experiments were carried out in a greenhouse (2017/2018 and 2018/2019) at Embrapa Soybean, in Londrina/PR, Brazil. A sample from the surface layer (0-20 cm) of a loam-textured *Latossolo Vermelho* (Typic Acrudox, USDA soil taxonomy), from an agricultural area containing an established population of soybean-nodulating rhizobia of 4.2×10^3 MPN.g^{-1} (most probable number per gram) (Vincent, 1970), was sieved (2 mm), arranged in trays (25 × 45 × 60 cm) with 40 kg each and air dried for 20 days.

The experimental design was completely randomized, with four replications in the first experiment and three replications in the second experiment, and the treatments were the times of exposure to dry soil. In the first experiment,

sowing was performed on 11/22/2017 and the inoculated seeds were exposed to dry soil for different times before irrigation: 0 (2 h), 1, 4, 11, 18, and 21 days. At the end of each time, irrigation was applied to induce germination, so that the plants of each treatment developed in different periods in the first experiment, according to each germination induction time. At 30 days before sowing, the seeds used had initial vigor of 76% by the tetrazolium test (França-Neto and Krzyzanowski, 2018) and germination power of 85% (Brasil, 2009). In the second experiment, the seeds were inoculated and sown with 0 (2 h), 1, 5, 12, 14, and 20 days before irrigation, which occurred on 11/30/2018, causing the inoculated seeds to be exposed to dry soil for the respective periods and the induction to germination to be synchronized to occur on the same day, so that the plants of each treatment developed in the same period. At 30 days before sowing, the seeds had initial vigor of 89% by the tetrazolium test (França-Neto and Krzyzanowski, 2018) and germination of 93% (Brasil, 2009).

Before setting up the treatments in the two experiments, the soybean seeds of the cv. BRS 511 were treated with fungicides (Pyraclostrobin and methyl thiophanate) and insecticide (Fipronil), at a dose of 2 mL of the commercial product.kg^{-1} of seeds. After 1 h of chemical treatment, the seeds were inoculated with inoculant containing the strains SEMIA 5079 of *Bradyrhizobium japonicum* and SEMIA 5080 of *B. diazoefficiens*, at the dose calculated to provide 1.2 × 10^6 cells per seed, with commercial inoculant containing in its formulation protective polymers (citric acid, xanthan gum, non-ionic surfactant, polyvinyl alcohol, mono- and dipotassium phosphate, saccharides and polysaccharides) to increase cell longevity (Hungria et al., 2020). In the first experiment, as sowing was performed at once, the recovery of *Bradyrhizobium* cells from the seeds to know the initial population was performed only once prior to sowing. In the second experiment, as sowing was performed on different days prior to induction of germination, cell recovery to know the initial population was performed at each sowing operation, because each of them required a new inoculation. In all cases, cell recovery before sowing was performed 2 h after inoculation, according to Brasil (2011a). At sowing, 250 seeds were distributed on the soil contained in the trays, in five grooves of 1 cm depth with 50 seeds each and then covered with a 1-cm-thick layer of the same soil.

During the experiments, the relative humidity and air temperature were monitored daily for 34 and 35 days with a data logger in the first and second experiments, respectively. Soil temperature was obtained at 2 cm depth after sowing, in the position in which the seeds were deposited, using probe thermometers (Figures 1A and B). After the first irrigation, at the end of each dry soil exposure period to induce germination, the trays were irrigated daily to maintain soil moisture at ~70% of the water retention capacity.

Immediately before the corresponding irrigation, at the end of each dry soil exposure period, three aliquots of 50 seeds were retrieved from the three central rows of each tray, suspended in 0.85% saline solution (NaCl), diluted in series of 1:10 up to 10^{-4}, and the dilutions 10^{-2}, 10^{-3}, and 10^{-4} were plated in triplicate on YMA medium (0.4 g.L^{-1} yeast extract, 5.0 g.L^{-1} mannitol, 0.5 g.L^{-1} dibasic potassium phosphate, 0.2 g.L^{-1} heptahydrate magnesium sulfate, 0.1 g.L^{-1} sodium chloride, 10-15 g.L^{-1} agar) containing 10% of Congo red solution at 0.25%, Actidione (667 µL.L^{-1} cycloheximide [84 µg.mL^{-1} in ethanol]) and vancomycin (333 µL.L^{-1} vancomycin hydrochloride [0.3 g.mL^{-1}]), for inhibition of contaminants from soil or seeds (Brasil, 2010; 2011a, 2011b). The results were expressed in Colony Forming Units (CFU) per seed (CFU.seed^{-1}).

The two remaining lateral rows were monitored for 11 days for emergence, to calculate the emergence speed index (ESI) and count the number of abnormal seedlings (Maguire, 1962). In the second experiment, cracks were observed in the cotyledons, which was accounted for and analyzed according to the treatments. After 11 days of monitoring, the seedlings were thinned to maintain 12 plants per tray, six equidistant in each remaining row.

At 35 days after emergence (DAE), the six plants of one of the rows were collected to evaluate nodulation (mass and number), shoot dry mass (SDM) and N concentration. For the quantification of SDM, the material was weighed after drying at 50 °C until reaching constant mass. Then, the total shoots (leaves, petioles and stems) were crushed in a Wiley mill, and sulfuric digestion was performed to determine the N concentration (Searle, 1984).

The six remaining plants were maintained until physiological maturity to evaluate yield components: number of

pods, total number of grains, 100-grain weight adjusted to 13% moisture, and oil and protein concentrations in grains, determined by the near infrared (NIR) method (Heil, 2010).

The data were subjected to analysis of variance by the F test ($p \leq 0.05$). The effects of the times of exposure to dry soil on the recovery of *Bradyrhizobium* cells from the seeds, compared with time zero (2 h), were evaluated using the two-tailed Dunnett's test ($p \leq 0.05$), with the data transformed to log(x+1). For the other variables, the means were compared by Duncan's test ($p \leq 0.05$), with data of the number of nodules transformed to $(x+1)^{1/2}$, while the other data were analyzed without transformation. All analyses were performed with Statistica software.

RESULTS AND DISCUSSION

The number of *Bradyrhizobium* cells recovered 2 h after inoculation, before exposure to dry soil, was 9×10^4 CFU.seed^{-1} in the first experiment (Figure 2A) and ranged from 8 to 9×10^4 CFU.seed^{-1} in the second experiment (Figure 2B). In the first experiment, 2 h after exposure to dry soil, cell recovery dropped to just over 3×10^4 CFU.seed^{-1} and remained stable until the 4th day, without differing from the number recovered after 2 h, but decreased dramatically in subsequent periods, when the maximum temperatures of the dry soil reached 50 °C (Figure 1A).

In the second experiment, the number of recovered cells, 2 h after dry soil exposure, was similar to that observed in the first, that is, about 3×10^4 CFU.seed^{-1} (Figure 2B). With one day under this condition, the number of cells was 2.5×10^4 CFU.seed^{-1}, without differing from 2 h of exposure on the previous day. However, with five days of exposure, the number dropped to about 7×10^3 CFU.seed^{-1}, with just over 6×10^2 CFU.seed^{-1} at 20 days. In both experiments, the longer the time of exposure of the seeds to dry soil, the greater the damage to survival of inoculated *Bradyrhizobium*.

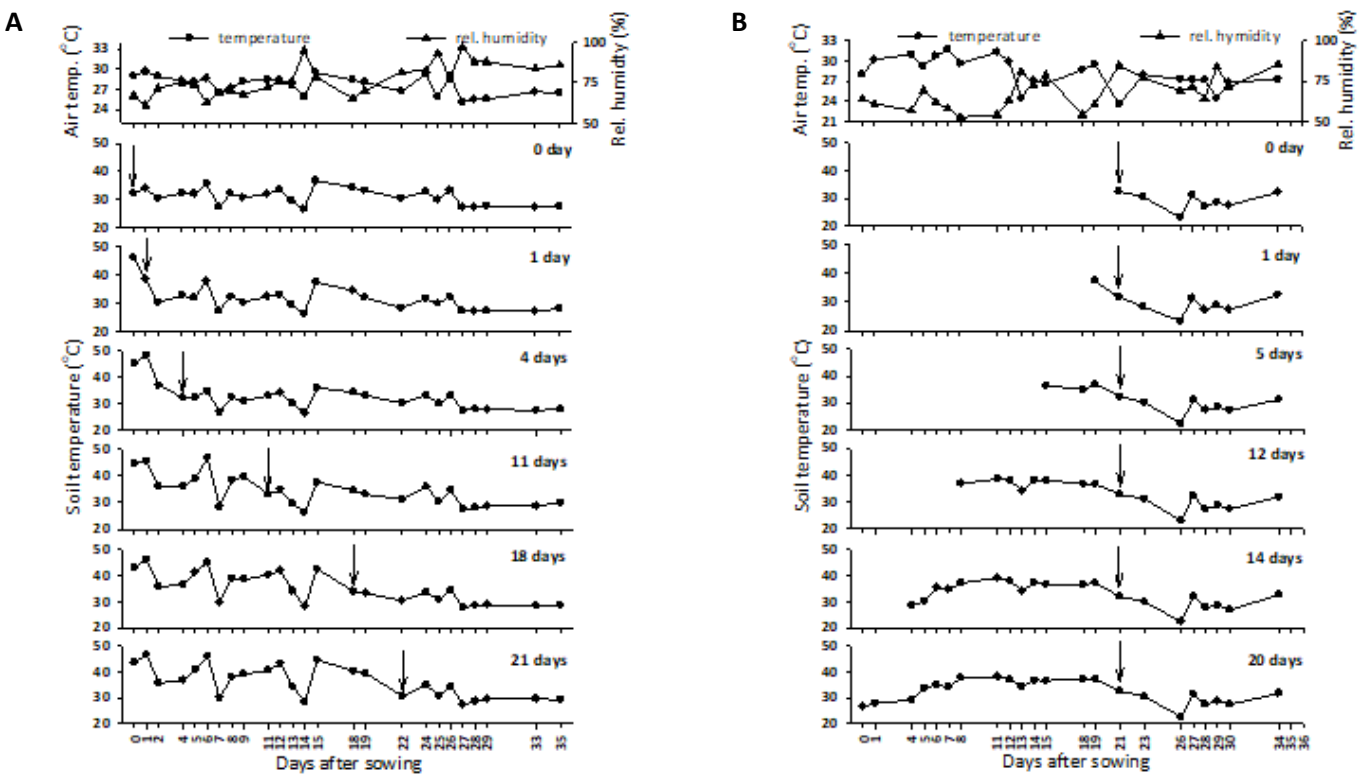

Figure 1. Air temperature and relative humidity, and soil temperature at 2 cm depth in the sowing trays at different times of exposure of soybean seeds inoculated with *Bradyrhizobium* spp. to dry soil, simulating the dry sowing condition in the first (A) in the second (B) experiment. Arrows indicate the moment of irrigation to induce germination.

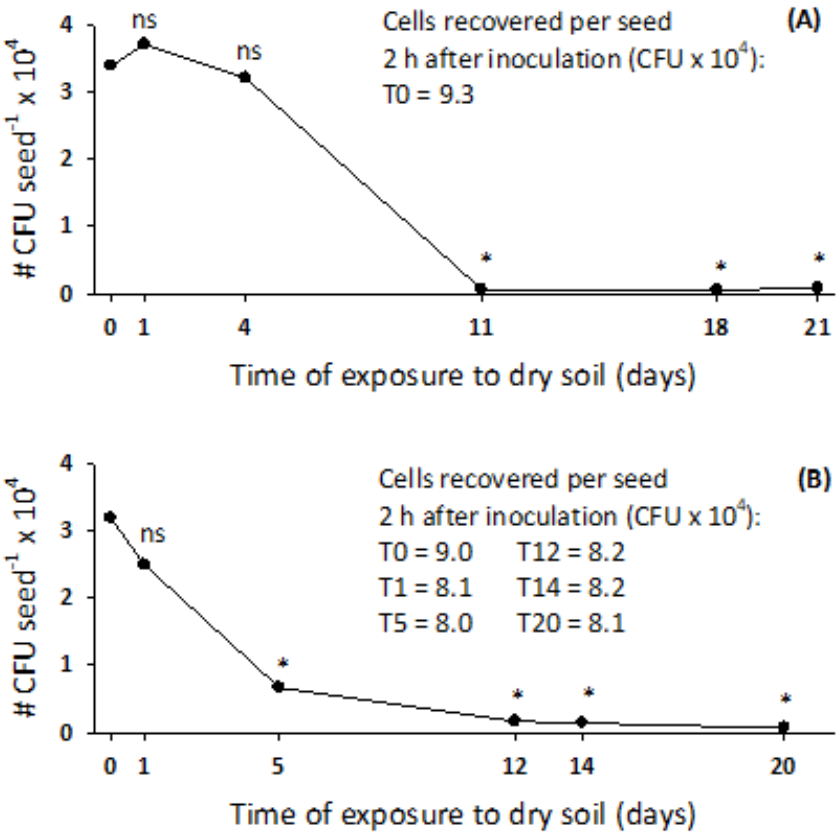

Figure 2. Number of *Bradyrhizobium* cells (CFU.seed^{-1}) recovered from soybean seeds 2 h after inoculation and at different times of exposure to dry soil, before irrigation, in the first (A) and in the second (B) experiment. * indicates significant difference ($p \leq 0.05$) compared with the recovery at 2 hours (zero time) by two-tailed Dunnett's test; ns = not significant. Data analyzed according to the transformation log (x+1).

The number of viable *Bradyrhizobium* cells in inoculated seeds influences the speed of nodulation and nodular occupation by the bacteria provided by the inoculant (Hungria et al., 2017). In areas already cultivated to soybean, where there is a population of soybean-nodulating rhizobia established in the soil, the inoculant dose is calculated to provide at least 1.2×10^6 cells per seed, so that at least 7-8%, that is, 8×10^4 to 1×10^5 viable cells, should be recovered at the time of sowing (Hungria et al., 2017). However, the number of recovered cells, with only 2 h of exposure, was already about one third of the minimum value indicated as ideal. This shows that even a short period of exposure to adverse conditions is enough to compromise the survival of *Bradyrhizobium* to levels below the ideal ones to promote rapid nodulation and high nodular occupation by elite inoculant strains (Hungria et al., 2017; Santos et al., 2019; Rodrigues et al., 2020).

Several factors influence the survival of bacteria inoculated in soybean seeds, and high temperatures and water restriction are among the most limiting (Cerezini et al., 2016). Cell death can also be aggravated by chemicals applied in the treatment of seeds (Santos et al., 2019; Hungria et al., 2020; Rodrigues et al., 2020), while the addition of cellular protective additives in the inoculant formulation may attenuate these adverse effects (Hungria et al., 2020). In the present study, despite the use of an inoculant with high cell concentration, above 5×10^9 CFU.mL^{-1} and with cell protectors, exposure to dry soil and temperatures above 32 °C, even in the short period of 2 h, caused a reduction in the number of viable *Bradyrhizobium* cells of at least 50%, which worsened over time and, after 11-12 days, it was only possible to recover just over 1,500 viable cells per seed, that is, about 50 times less than the desirable minimum (Hungria et al., 2017).

The effect of thermal and water stress on *Bradyrhizobium* cells can be aggravated by the chemical treatment of seeds, which occurs not necessarily through the toxicity of the active ingredient, but may occur due to constituents of the formulation, such as dyes and preservatives, high salinity, and pH extremes (Campo et al., 2009). In the laboratory, there was up to 98% reduction of *Bradyrhizobium* cells in seeds treated with incompatible fungicides; in the field, soybean nodulation decreased 14% in soils with an established *Bradyrhizobium* population and 70% in soil without an established population due to incompatibility between inoculant and chemical treatment (Campo et al., 2009). In areas with an established population, even if there is reduction in the number of cells recovered from the seeds, there will be, though delayed, nodulation by soil rhizobia, which can still support good levels of yield. However, when inoculation is performed following good practices, which favor the survival of inoculated bacteria, the yield gains are 8% in average (Hungria et al., 2017).

The negative effects of sowing on dry soil were not limiting only to inoculation, but the exposure of seeds to temperatures above 40 °C in the soil, with peaks of 50 °C in the hottest hours (Figure 1), also reduced the ESI of the seedlings as the exposure time increased (Table 1). In the first experiment, which used a lot of seeds with initial vigor of 76% and germination of 85%, the exposure time did not interfere in germination, which remained around 60% at all times of exposure to dry soil. However, the number of abnormal seedlings increased by more than three times, from 7% with 2 h to 24% with 21 days of exposure (Table 1). In turn, the ESI decreased from 19.5 to 12-13, already from the fourth day of exposure, a value that remained until the 21st day (Table 1). In the second experiment, in which the seed lot had initial vigor of 89% and germination of 93%, there was no significant effect on the number of abnormal seedlings, which ranged from 1 to 3%, or on the germination rate, which varied between 89 and 78% between the shortest and the longest exposure time. The occurrence of cotyledons with cracks was found only in the second experiment. These symptoms increased with the time of exposure of seeds to dry soil, from 1% with 2 h to ~50% from 5 days onwards (Table 1). The ESI in the second experiment decreased from 37.8 at time zero to 13.8 on the 20th day of exposure, with significantly lower values on the 5th day and even lower values from the 12th day onwards, remaining at this level until the 20th day.

The initial vigor of the seeds has an effect on the originated seedlings and the exposure of seeds with lower vigor to adverse conditions increases the occurrence of abnormal seedlings, which generate dominated plants with lower production potential (Dias et al., 2011). In the second experiment, in which seeds with greater vigor were used, exposure

Table 1. Germination (%), abnormal seedlings (%), and emergence speed index (ESI) according to the time of exposure of soybean seeds to dry soil.

First Experiment	Time of exposure to dry soil					
	0 h	1 d	4 d	11 d	18 d	21 d
% Germination	61 ^{ns}	50	56	64	57	63
% Abnormal seedlings	7 b	6 b	8 b	11 b	13 b	24 a
ESI	19.5 a	15.8 ab	12.2 bc	13.0 bc	12.5 bc	12.0 c
Second Experiment	0 h	1 d	5 d	12 d	14 d	20 d
% Germination	89 ^{ns}	83	83	75	78	78
% Abnormal seedlings	2 ^{ns}	3	2	3	2	1
% Cracked cotyledons $^{(a)}$	1 c	17 b	50 a	50 a	42 a	45 a
ESI	37.8 a	35.6 a	23.1 b	16.8 c	14.2 c	13.8 c

Means followed by equal letters do not differ from each other by Duncan's test ($p \leq 0.05$); ns = not significant.
$^{(a)}$ cracked cotyledons were not observed in the first experiment.

to dry soil did not increase the occurrence of abnormal seedlings. When other adverse factors were evaluated, such as the high volume of solution in the treatment of soybean seeds, the ones with high vigor showed higher physiological quality than those with low vigor, and higher volumes of solution reduced the physiological quality of low-vigor seeds, which emphasizes the importance of using seeds of higher vigor to originate plants with higher production potential, even under adverse conditions (Brzezinski et al., 2017).

Regarding the crack of cotyledons in seedlings in the second experiment, according to one of the rare research studies dealing with this subject, exposure to high temperatures during seed imbibition can cause such lesions. In fact, seeds exposed to dry soil for five days or more were also exposed to temperatures above 40 °C (Figure 1B). However, the occurrence of cracks in cotyledons may vary with the origin of the seeds and with the conditions under which they were produced, manipulated, and stored (Sorrells and Pappelis, 1976). This helps explain the fact that, despite using seeds of the same variety, cracks were observed in the cotyledons only in the seedlings of the second experiment.

Seeds that remained for the shortest time in the dry soil, especially those induced to germinate 2 h after sowing or the next day, were the ones with the highest ESI values. In addition, the seeds with lower vigor used in the first experiment showed lower ESI values, while those in the second experiment, with higher vigor, showed higher indices. However, from the fourth/fifth day of exposure, the indices decreased significantly, compared with the zero time (0 h), worsening as the exposure time increased. Again, the importance of using seeds of higher vigor, with higher ESI, was evidenced (Brzezinski et al., 2017). As the time of exposure of the seeds to dry soil increased, there was a delay in ESI in both experiments, suggesting damage to their physiological quality.

Although germination at the end of 11-12 days showed little variation (50-64% in the first and 75-89% in the second experiment), with no significant effect of dry soil exposure time (Table 1), there was a delay in the percentage of emerged seedlings with the increase in the time the seeds remained exposed to dry soil (Figure 3). High temperatures and water restriction limit the crop development, mainly in germination-emergence and flowering-grain filling. In order to have uniformity in the establishment (stand) of plants, the seed needs to absorb water, at least 50% of its mass to germinate (Mohammadi et al., 2012). Although there was no effect of the time of exposure of seeds to dry soil on germination, there was an evident delay in the emergence of seedlings whose originating seeds were exposed to dry soil for four and five days onwards in the first (Figure 3A) and second (Figure 3B) experiment, respectively. The delay in seedling emergence speed indicates a loss of vigor of the seeds (Abati et al., 2020), which may reduce the yield potential of the crop. According to Seixas et al. (2020), the ideal soil temperature for soybean sowing is 25 °C, which promotes rapid emergence and uniformity of seedlings.

In addition to the decrease in the number of viable *Bradyrhizobium* cells inoculated in seeds exposed to dry soil, the limited exchange of molecular signals between bacteria and seedlings grown from physiologically less active seeds may also limit the initial stage of the symbiosis establishment, which also contributes to decrease the crop yield potential (Hungria et al., 2017).

The exposure time to dry soil significantly decreased the 100-grain weight in the first experiment, but not in the second (Figure 4). However, this decrease in the first experiment may not have been a consequence of the damage to seed physiology due to the exposure to dry soil, but probably resulted from the fact that plants originated from seeds exposed to dry soil for longer were induced to germinate later, according to the dry soil exposure treatment, and originated plants developed in a period with ever shorter days. When the seeds were induced to germinate simultaneously in the second experiment, the plants of all treatments developed at the same time and there was no effect on the 100-grain weight, which reinforces the hypothesis that the decrease in the 100-grain weight of plants originated from seeds that remained longer in the dry soil was due to the decrease in day length and not necessarily to the loss of physiological quality of the seeds.

Treatments did not influence the variables shoot dry mass, shoot nitrogen concentration, number of nodules, mass of nodules, number of pods, number of grains, mass of grains per plant, and protein and oil concentrations in the grains in both experiments (Table 2). Under field conditions, periods of drought and high temperatures negatively impact

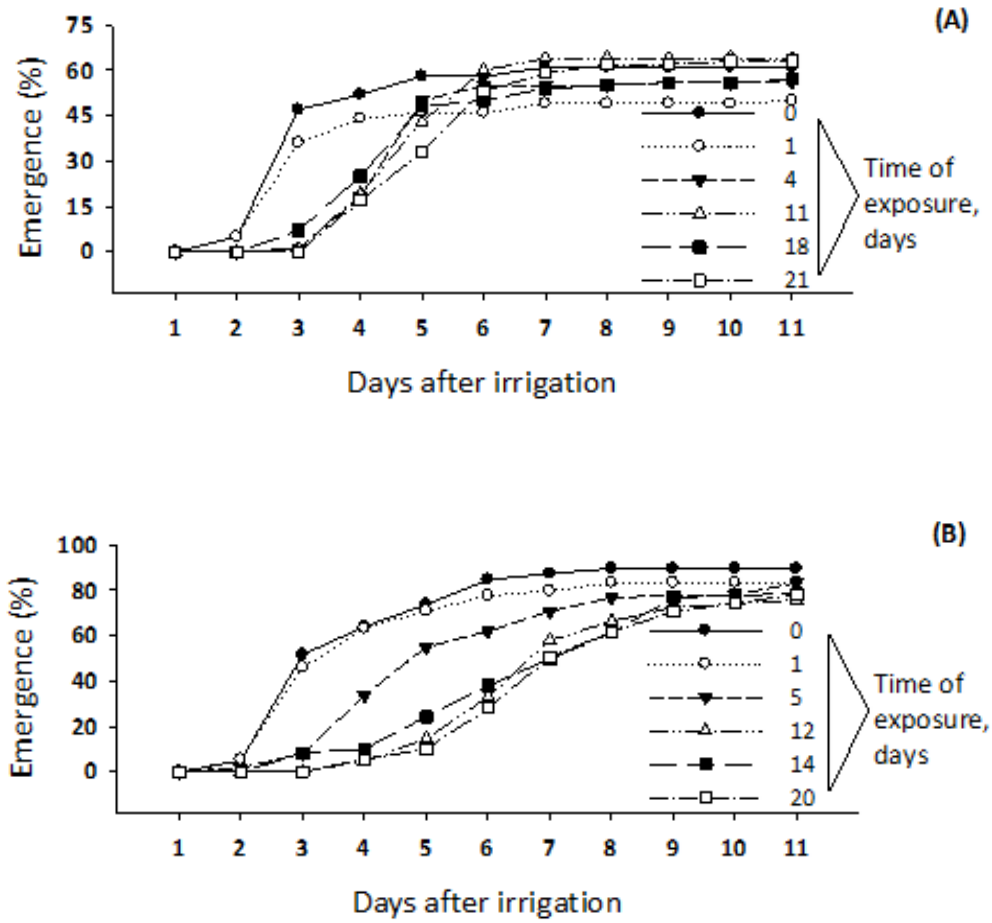

Figure 3. Emergence of soybean seedlings (%) over 11 days after irrigation, according to the time of exposure of seeds to dry soil before irrigation, in the first (A) and in the second (B) experiment.

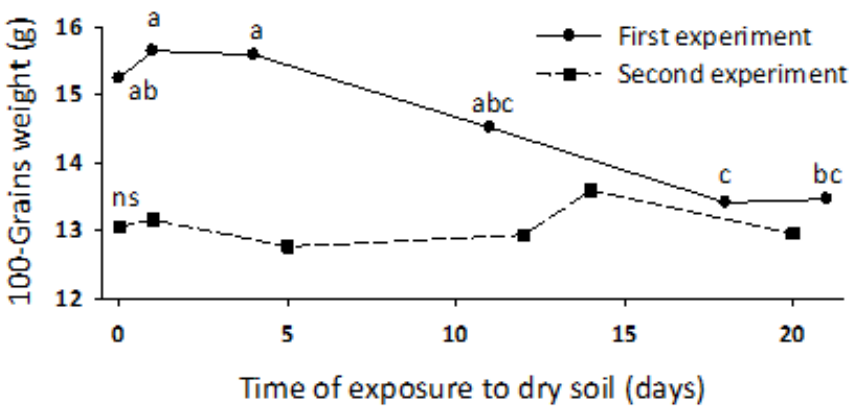

Figure 4. 100-grains weight of soybean at 13% moisture, according to the time of exposure to dry soil of seeds that originated the plants. Means followed by equal letters do not differ from each other by Duncan's test ($p \leq 0.05$); ns = not significant.

Table 2. Shoot dry mass (SDM), shoot nitrogen concentration, number of nodules, mass of nodules, number of pods, number of grains, mass of grains per plant, and oil and protein concentrations in soybean grains, according to the time (days) of exposure to dry soil.

Days of exposure	SDM ($g.pl^{-1}$)	N ($g.kg^{-1}$)	Nº of Nod.	Mass of Nod. ($mg.pl^{-1}$)	Nº of $pods.pl^{-1}$	Nº of $grains.pl^{-1}$	M. grains ($g.pl^{-1}$)	Oil (%)	Proteins (%)
First experiment									
0 (2 h)	2.1 ^{ns}	19.7 ^{ns}	49.9 ^{ns}	226 ^{ns}	13.7 ^{ns}	29.1 ^{ns}	4.6 ^{ns}	24.3 ^{ns}	35.7 ^{ns}
1	2.2	18.1	43.8	208	13.8	30.8	5.0	25.0	35.3
4	2.1	20.7	59.2	224	13.5	30.3	4.7	24.9	36.3
11	2.0	20.6	54.1	252	15.4	34.9	5.0	25.2	34.6
18	2.0	17.8	45.2	170	16.7	36.6	4.9	25.2	36.0
21	2.3	17.7	45.9	213	18.7	35.4	5.0	25.3	36.2
Second experiment									
0 (2 h)	2.1 ^{ns}	25.8 ^{ns}	35.4 ^{ns}	146 ^{ns}	37.4 ^{ns}	75.5 ^{ns}	4.9 ^{ns}	24.7 ^{ns}	33.2 ^{ns}
1	2.3	28.5	36.0	143	39.6	74.9	5.5	24.1	35.2
5	1.9	21.7	26.6	112	37.8	73.0	5.2	24.8	33.8
12	2.2	26.3	33.0	122	38.1	77.6	4.7	24.2	34.9
14	2.1	21.7	37.6	133	37.7	78.8	5.6	24.4	32.2
20	2.2	23.3	33.0	125	35.3	67.7	5.4	25.5	34.8

ns = not significant by F test ($p > 0.05$).

soybean plants, in terms of germination, initial development, symbiosis establishment and BNF, with negative effects on yield (Cerezini et al., 2016), as well as stressful conditions for seeds such as early treatment with chemicals and storage at inadequate temperatures (Brzezinski et al., 2015; Abati et al., 2020). However, in the present study, the variables evaluated were not influenced by the exposure to dry soil. This can be attributed to the fact that the plants were grown with adequate water supply throughout their development after germination, under conditions conducive to nodulation and plant development, even though there was a negative effect on *Bradyrhizobium* survival and physiological quality of seeds due to the exposure to dry soil. The population of *Bradyrhizobium* cells in the soil used in the assay was 4.2×10^3 $MPN.g^{-1}$, which is sufficient to promote good nodulation under ideal conditions, even in treatments in which the recovery of cells from the seeds was hampered by the exposure to dry soil. Under field conditions, water restriction reduces nodulation, with losses to BNF and crop yield potential (Cerezini et al., 2016). This result would probably be different if the plants had been grown under field conditions, since a compromised initial development hampers the final result under these conditions, especially in years with irregular rainfall distribution. Conducting the experiments in greenhouse was essential to control the exact moment and amount of water to be supplied to induce germination, which is not always possible in the field. However, the negative effects of the initial exposure of seeds and inoculated bacteria to stress did not affect the subsequent plant development in the greenhouse. These results emphasize that tests performed under controlled conditions to evaluate stressful conditions to *Bradyrhizobium* or soybean seeds do not always reflect the plant responses that would occur in the field and, therefore, need to be interpreted with caution.

CONCLUSIONS

Sowing in dry soil is detrimental to *Bradyrhizobium* survival, leading to a recovery of viable cells with values below that recommended by research.

Sowing in dry soil reduces the physiological quality of the seeds and the emergence speed index as the time of exposure of the seeds increases.

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