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Article

Residual Effect of the Herbicide Glyphosate on the Germination of Bean Seeds Under Field and Controlled Conditions

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RESUMO

O glifosato geralmente resulta em baixo efeito residual no campo. No entanto, em condições específicas de ambiente controlado ou a campo, as doses podem ser determinantes e impactar na germinação e no desenvolvimento inicial de sementes de feijão. Diante do exposto, o presente estudo teve como objetivo avaliar, tanto em condições naturais de cultivo (campo), como em condições controladas (laboratório) a interferência do residual do herbicida glifosato sobre a germinação de plântulas de feijão preto em diferentes intervalos de aplicação. Foram avaliados os intervalos de 21 dias (T1), 14 (T2), 7 (T3) e 0 dias (T4) antes da semeadura, além do Controle (TC), sem aplicação. Analisou-se a Taxa de Germinação (TG), Velocidade de Emergência (VE) e Índice de Velocidade de Emergência (IVE). A campo, apenas VE apresentou diferença significativa (TC, T2, T4 vs. T1, T3), sugerindo influência de fatores ambientais, como a umidade do solo. Em ambiente controlado, os resultados evidenciaram efeito temporal, em que o T4 (aplicação no dia da semeadura) apresentou os menores valores, com redução significativa em todos os parâmetros, enquanto TC e T1 (aplicação mais antecipada) destacaram-se com os maiores valores, o que permite inferir uma possível degradação do glifosato ao longo do tempo e a redução do seu impacto fitotóxico. Conclui-se que aplicações próximas à semeadura comprometem a germinação, reforçando a necessidade de intervalos seguros.

Palavras-chave: *Phaseolus Vulgaris*; herbicida; dessecação; leguminosa.

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ABSTRACT

Glyphosate generally results in a low residual effect in the field. However, under specific conditions in a controlled environment or in the field, the doses can be decisive and impact the germination and early development of bean seeds. In light of the above, the present study aimed to evaluate, both under natural cultivation conditions (field) and controlled conditions (laboratory), the interference of residual glyphosate herbicide on the germination of black bean seedlings at different application intervals. The intervals evaluated were 21 days (T1), 14 (T2), 7 (T3), and 0 days (T4) before sowing, in addition to the Control (TC), with no application. Germination Rate (GR), Emergence Speed (ES), and Emergence Speed Index (ESI) were analyzed. In the field, only VE showed a significant difference (TC, T2, T4 vs. T1, T3), suggesting the influence of environmental factors, like the soil moisture. In a controlled environment, the results showed a temporal effect, with T4 (application on the day of sowing) presenting the lowest values, with a significant reduction in all parameters, while TC and T1 (earlier application) stood out with the highest values, which allows us to infer a possible degradation of glyphosate over time and a reduction of its phytotoxic impact. It is concluded that applications close to sowing compromise germination, reinforcing the need for safe intervals.
Keywords: *Phaseolus Vulgaris*; herbicide; desiccation; legume.

Introduction

Common bean (Phaseolus vulgaris L.) is a culturally established staple food in Brazil (Silva et al. 2016). The country has global relevance as the only commercial-scale producer of carioca beans, which represent approximately 40% of total national production, while other types, notably black beans, account for the remaining 60%. In addition, beans show high domestic consumption, being present in more than 70% of Brazilian households, thus constituting a crop of major importance in the Brazilian agricultural scenario (IBRAFE 2018).

Beans are a short-cycle crop, which allows up to three harvests per year, and are cultivated throughout the national territory in different regions and sowing periods, generally during the soybean off-season. This enables crop rotation with other species, diversifying production and providing additional income options for farmers (Silva et al. 2016). Despite its importance, yield levels are often low and are related to several factors, such as market conditions, irregular climatic patterns, inadequate use of inputs, phytosanitary problems, and inappropriate management practices. In this context, proper soil preparation, especially pre-planting methods, is of great importance for good crop performance during the initial growth stages (Lopes & Faria 1995; Nova 2019).

Considering the nutritional and economic relevance of bean production in Brazil and the internal constraints faced by the sector, it becomes necessary to adopt investments in technological improvement through agricultural implements, with the aim of increasing crop productivity and minimizing risks and costs (Ramos et al. 2022). In this sense, the use of pesticides has become one of the most common tools in agricultural systems, and herbicides play an essential role in production technology (Silva et al. 2012).

Among the main herbicides used for pre-sowing desiccation, glyphosate-based formulations are the most widely adopted (EMBRAPA 2005), as they interfere with weed flowering and block nutrient absorption. Due to its versatility and high efficiency, glyphosate acts rapidly and controls a wide range of weed species, in addition to presenting low toxicological risk to animals, which contributes to its widespread use in agriculture (Gomes et al. 2020).

Glyphosate (N-(phosphonomethyl)-glycine) is a systemic herbicide with conditional selectivity (selective for genetically modified crops with RR technology), applied both pre- and post-emergence in several crops, including common beans. Its mechanism of action involves inhibition of the enzyme EPSPs (5-enolpyruvylshikimate-3-phosphate synthase) in the shikimic acid metabolic pathway, preventing the synthesis of essential amino acids required for plant growth (Ribeiro et al. 2023). In addition, glyphosate shows strong adsorption to clay-rich soils, resulting in low leaching and reduced soil mobility, as well as high water solubility and low volatility (Villa et al. 2019).

Studies evaluating different glyphosate doses applied during pre-sowing have reported interference with seed germination in certain species, indicating reduced germination percentages when exposed to the active ingredient (Silva et al. 2012). However, few studies have evaluated the effect of application timing before sowing, although available evidence indicates inhibition of germination and early seedling development when the interval between application and sowing is short (Rocha Filho 2021).

Seed germination can be influenced by several factors, such as light, temperature, relative air humidity, substrate, and soil water availability. Among these, water availability is considered the most significant, due to its role in embryonic imbibition, metabolic reactivation, and reserve mobilization from cotyledons. During this stage, the presence of non-natural chemical substances in the soil solution may inhibit or stimulate germination depending on their nature and concentration (Santos et al. 2021).

Several studies have demonstrated that glyphosate exerts harmful effects on the physiological potential of seeds lacking resistance genes, causing phytotoxicity and poor seed performance (Daltro et al. 2010). Duke et al. (1979) observed that conventional soybean seeds exposed to glyphosate may initiate germination, but subsequent development becomes negligible and eventually ceases.

The indiscriminate use of glyphosate has been simulated in several experiments aiming to better understand how the active ingredient affects crop sprouting and early growth stages, with most studies conducted under controlled greenhouse conditions (Silva et al. 2012). However, under field conditions, little is known about the interference caused by this herbicide, and there is still disagreement among researchers, likely due to the presence of uncontrolled environmental variables (Silva et al. 2012; Melhorança Filho et al. 2011).

Given the scarcity of studies addressing the effects of pre-sowing application and the residual activity of glyphosate on bean seeds and seedlings, the present study aimed to investigate, under both natural cultivation conditions (field) and controlled conditions (laboratory), the residual interference of glyphosate on the germination of black bean seedlings at different application intervals.

Methodology

Two complementary experiments were conducted: one under field conditions and another under laboratory conditions, using standardized experimental design and treatments.

Experiment conducted under natural cultivation conditions

The field experiment was carried out on a rural property located in the district of Feijão Miúdo, in the municipality of Três Passos, RS, Brazil (27°26'02.2" S, 53°56'43.4" W). The soil of the area was classified as a Red Latosol (Santos et al. 2018), characterized as deep, well-drained, friable, with a clayey texture (more than 60% clay) and a small sand fraction (less than 10%).

As a preliminary step prior to the installation of the experiment, the area, which was under fallow conditions, underwent initial preparation through mechanical removal of spontaneous vegetation using a brush cutter. Subsequently, the experimental design was demarcated, consisting of 20 plots measuring 3 m² each (2 m × 1.5 m), spaced 0.5 m apart to avoid contamination between treatments. The total area occupied by the experiment was 77.43 m², including plots and isolation corridors.

The interference of the herbicide on bean seed germination was evaluated at four different pre-sowing application times: Control treatment (TC), with no herbicide application; T1: 21 days before sowing (21 DPS); T2: 14 days before sowing (14 DPS); T3: 7 days before sowing (7 DPS); and T4: 0 days before sowing (0 DPS). Each treatment consisted of four replications, arranged in a randomized complete block design (RCBD).

The herbicide used was ZAPP QI 620, registered with the Brazilian Ministry of Agriculture under number 12908. The applied dose followed the manufacturer's label recommendation, at 2.8 L ha^{-1}, indicated for annual weed species with difficult control, which characterized the experimental area. Applications were performed using a 20 L backpack sprayer during the coolest hours of the day.

Herbicide applications were carried out 21, 14, 7, and 0 days before sowing, which was performed according to the Agricultural Zoning for the bean crop (MAPA 2022). Sowing was performed manually, with 40 cm spacing between rows, totaling five rows per plot, and an average plant population of 15 plants per linear meter, corresponding to 75 plants per plot. No herbicide application was performed in the Control treatment (TC); however, mechanical vegetation removal was conducted to allow sowing.

The evaluated parameters were Germination Rate (TG), Emergence Speed Index (IVE), and Emergence Speed (VE).

TG was determined by counting the number of emerged seedlings considered normal at 24 days after sowing. Normal seedlings were defined as those capable of developing into normal plants under favorable conditions (Brasil 2009). VE and IVE were also evaluated, representing, respectively, the average number of days required for emergence and the average number of normal seedlings emerged per day (Eicholz et al. 2012).

VE was determined by counting emerged seedlings at 15, 18, 21, and 24 days after sowing. IVE was calculated using the equation proposed by Maguire (1962): $IVE = (G1/N1) + (G2/N2) + \dots$, where IVE is the Emergence Speed Index, G is the number of normal seedlings in each count, and N is the number of days after sowing. Based on IVE values, VE was calculated using the equation proposed by Edmond & Drapala (1958): $VE = [(N1G1) + (N2G2) + \dots + (NnGn)] / (G1 + G2 + \dots + Gn)$, where VE is the Emergence Speed (days).

Experiment conducted under controlled conditions

The controlled experiment was conducted in a biochemical oxygen demand (BOD) incubator at the laboratory of the Universidade Estadual do Rio Grande do Sul, Três Passos campus. The same experimental treatments and parameters described for the field experiment were applied. Seeds were germinated in a BOD incubator set at 25 °C with a 12-hour photoperiod, using sieved sand (1.8 mm mesh), sterilized at 120 °C for 24 hours, following the Rules for Seed Analysis (RAS).

Each replication was conducted in disposable trays (size no. 10), containing 320 g of sand. Thirty bean seeds were evenly distributed in each tray. Herbicide applications were carried out 21, 14, 7, and 0 days before sowing. After sowing, trays were irrigated to simulate field conditions, and TG, VE, and IVE were evaluated using the same methodology described for the field experiment.

Data obtained from both experiments were subjected to analysis of variance (ANOVA) to verify statistical assumptions, followed by Tukey's test for comparison of means at a 5% significance level ($p < 0.05$). Statistical analyses were performed using the Sisvar software (Ferreira 2019).

Results and Discussion

Germination Rate, Emergence Speed, and Emergence Speed Index of bean seeds under field conditions

In the experiment conducted under field conditions, no statistically significant differences were observed among treatments for the Germination Rate (TG), although a tendency toward higher germination was noted in treatments without herbicide application or when application occurred further in advance of sowing. The Control treatment (TC) showed the highest germination rate (69%), followed by T1 (62%) and T2 (61.3%). Treatment T3 resulted in 40% germination, whereas T4 (application on the day of sowing) showed an unexpected increase, reaching 53.3% (Table 1).

Table 1 - Germination Rate (GR), Emergence Speed (ES), and Emergence Speed Index (ESI) in bean cultivation under field conditions, Três Passos-RS

Treatment	TG (%)	VE (days)	IVE[1]
TC	69,0 a	19,9 a*	8,4 a
T1	62,0 a	27,7 b	8,6 a
T2	61,3 a	20,1 a	7,8 a
T3	40,0 a	28,3 b	7,6 a
T4	53,3 a	20,2 a	7,3 a

TC: Control; T1: 21 days before sowing; T2: 14 days before sowing; T3: 7 days before sowing; T4: 0 days before sowing. CV: 14.76%. Means followed by the same letter do not differ from each other, according to Tukey's test at 5%.

For Emergence Speed (VE), treatments TC (Control), T2 (14 DPS), and T4 (0 DPS) showed statistically significant differences in comparison with T1 (21 DPS) and T3 (7 DPS) (Table 1). According to Edmond and Drapala (1958), higher VE values reflect greater physiological potential of seeds, since rapid emergence is directly associated with seed vigor. However, for the Emergence Speed Index (IVE), calculated according to the equation proposed by Maguire (1962), no statistically significant differences were observed among treatments (Table 1).

This dissociation between VE and IVE is relevant, because although IVE, despite being dimensionless, is frequently correlated with the average number of normal seedlings emerging per day and interpreted as an indicator of vigor (Borghetti & Ferreira 2004), in this study it did not reflect the variations detected in VE. This phenomenon is consistent with historical criticisms regarding the limitations of IVE in discriminating subtle differences in vigor, as observed by Brown and Mayer (1986) in seeds of other species, and corroborates the findings of Ávila et al. (2005) in canola, reinforcing the need for joint analysis of multiple parameters for a robust evaluation of seed vigor.

When analyzing the results for the Emergence Speed Index (IVE), even in the absence of statistical differences, it is possible to observe that the values followed a logical trend associated with the application intervals, showing higher values in treatments with longer intervals between herbicide application and sowing. This pattern was also reported by Rocha Filho (2021), who evaluated pre-sowing application of herbicides (2,4-D + glyphosate) in cowpea (Vigna unguiculata), observing a positive influence of wider intervals on seed vigor.

The Control treatment (TC) showed the best performance for VE, corroborating studies such as Silva et al. (2012), who observed higher emergence speed in soybean seeds not exposed to herbicides. Treatment T2 (14 DPS) also showed superior VE, reinforcing evidence that desiccation carried out approximately two weeks before sowing is associated with good early development of annual crops (Bervald et al. 2010). This interval reduces the risk of interference from residual glyphosate in the soil and minimizes problems such as herbicide drift and transfer between plants through the root system (Bervald et al. 2010; Rodrigues et al. 1982; Yamada & Castro 2007).

However, the results observed for treatment T4 (0 DPS) were contradictory. Despite desiccation performed immediately before sowing, its VE performance was comparable to that of the Control treatment (Table 1), differing from the findings of Santos et al. (2007), who reported lower performance in soybean crops when desiccation was carried out close to sowing. In addition, in the present study, treatments T4 (0 DPS) and T2 (14 DPS outperformed T1 (21 DPS) and T3 (7 DPS), which also contrasts with the same study, in which applications performed 7 and 21 days before sowing resulted in better plant development compared to those carried out 15 days before sowing or on the day of planting.

Germination Rate, Emergence Speed, and Emergence Speed Index of bean seeds under controlled conditions

Under laboratory conditions, statistically significant differences were observed for Germination Rate (TG) (Table 2). A marked variation among treatments was observed, especially between T1 (21 DPS) and T4 (0 DPS). The Control treatment (TC) showed the highest germination rate (90.9%), followed by T1 (85.5%), T2 (81.7%), and T3 (78.8%). The lowest value was recorded for T4 (52.7%), which differed statistically from the other treatments (Table 2).

Table 2. Germination rate (GR), emergence speed (ES), and emergence speed index (ESI) in bean cultivation under controlled conditions, Três Passos-RS.

Treatment	TG (%)	VE (days)	IVE[1]
TC	90,9 a	42,3 b	4,5 a*
T1	85,5 a	39,5 b	4,6 a
T2	81,7 a	37,1 ab	4,6 a
T3	78,8 ab	36,4 ab	4,6 a
T4	52,7 b	24,3 a	4,5 a

TC: Control; T1: 21 days before sowing; T2: 14 days before sowing; T3: 7 days before sowing; T4: 0 days before sowing. [1]IVE: related to the average number of normal seedlings emerging each day. *Averages followed by the same letter in the columns do not differ from each other, according to Tukey's test at 5%.

These TG results corroborate previous studies, such as Rocha Filho (2021), who identified a reduction in the phytotoxic effects of herbicides as the interval between application and sowing increased. Similarly, Bervald et al. (2010) reported better performance in soybean cultivars when herbicide application was carried out with greater advance. These results may be explained by the hypothesis proposed by Nagata et al. (2000), according to which glyphosate induces deficiency of aromatic amino acids and phenolic compounds, inhibiting organ formation and root differentiation, resulting in reduced germination.

In addition, the reduction in the negative impact of glyphosate on seed germination observed with longer intervals between application and sowing may be attributed to herbicide–soil interactions. After application, glyphosate is rapidly adsorbed, reducing its availability in the soil solution and, consequently, its herbicidal activity over time (Moraes & Rossi 2010; Toni et al. 2006). Microbial degradation also plays an important role in this process, with approximately 50% of the molecule being metabolized within 28 days and up to 90% within 90 days (Rodrigues & Almeida 2005).

However, an atypical behavior was observed for treatment T4 (0 DPS) in the field experiment (Table 1), which showed an increase in TG compared to T3. Herbicide volatilization may be a plausible explanation for this result, due to climatic conditions during the experimental period, characterized by high temperatures and absence of rainfall, which favor evaporation and reduce glyphosate activity (EMBRAPA 2005). Furthermore, considering that adsorption is a dynamic physicochemical process involving herbicide–soil–water interactions, and given unfavorable environmental conditions, it can be presumed that herbicide molecules remained retained in dry soil, without being absorbed by the seeds during the imbibition process in T4 (0 DPS). According to Moraes and Rossi (2010), approximately 90% of applied glyphosate is sorbed by the soil almost instantaneously, with the remaining fraction being adsorbed within minutes.

Under controlled laboratory conditions, the results obtained for Emergence Speed (VE) differed from those observed in the field experiment. For this parameter, mean values followed the same pattern observed for TG, with TC (Control) and T1 (21 DPS) showing statistically significant differences in comparison with T2 (14 DPS) and T3 (7 DPS), while T4 (0 DPS) maintained the lowest VE value (Table 2). These results indicate that, under controlled conditions, desiccation performed closer to sowing intensifies the phytotoxic effects of glyphosate, delaying seedling emergence. However, similarly to the field experiment, no statistically significant differences were observed for the Emergence Speed Index (IVE) among treatments (Table 2).

The results of this study are consistent with those reported by Rocha Filho (2021), who evaluated the residual activity of herbicides in cowpea (Vigna unguiculata). The author observed that glyphosate significantly affected TG, VE, and IVE, indicating that shorter intervals between application and sowing resulted in lower emergence speed and vigor indices, with no statistical differentiation observed after a 21-day interval. According to the author, phytotoxic effects are diluted over time due to degradation of the glyphosate molecule, which reduces its interference with seedling elongation and cell division as its concentration in the cultivation medium decreases.

Glyphosate acts on the enzyme EPSPs (5-enolpyruvylshikimate-3-phosphate synthase), which operates in the chloroplast. Inhibition of this enzyme by the herbicide blocks the synthesis of three aromatic amino acids—tyrosine, phenylalanine, and tryptophan—which are essential for plant growth, defense, development, and protein production, among other functions (Velini et al. 2009). As a result, symptoms include growth arrest within a few hours after application, followed by leaf yellowing and slow plant death within one to three weeks, due to the systemic action of glyphosate (Marchi et al. 2008).

According to Taiz and Zeiger (2009) and Barbosa et al. (2017), glyphosate influences the formation of several compounds and causes damage to the germination process of maize seeds. One notable effect is the imbalance between indole-3-acetic acid (IAA) and abscisic acid (ABA), with glyphosate-induced reductions leading to increased inhibition of germination due to higher ABA levels. Duke et al. (1979) and Melhorança Filho et al. (2011) observed that, in the presence of glyphosate, conventional soybean seeds initiate germination; however, subsequent development becomes negligible, resulting in abnormal seedlings that eventually stop growing due to glyphosate interaction with the enzyme α-amylase. According to Bewley (1994), this enzyme is responsible for breaking down starch into smaller molecules to supply energy for plant development and is activated by moisture and temperature.

Bertoncelli et al. (2018) reported that root length is affected by glyphosate action, due to reduced production and translocation of auxins and decreased gibberellin levels, which negatively affect germination and vegetative development. However, in the present study, the root system was not evaluated.

Silva et al. (2009), when evaluating lower doses of glyphosate in sugarcane and common bean, described a phenomenon known as the hormetic effect. This effect occurs when a substance, at low doses, stimulates growth or biological activity rather than inhibiting it. In the context of glyphosate, this suggests that in treatments with application close to sowing (0 DAS), the herbicide may begin to decompose while still maintaining a relevant concentration in the soil, which could stimulate plant growth. According to Velini et al. (2009), stimulation at low dosages is associated with reduced interaction between glyphosate molecules and the EPSPs enzyme compared with higher concentrations. Glyphosate does not act on plants at doses between 0 and 1.8 g ha^{-1}. Above this level, stimulatory effects may occur, while doses between 7.2 and 36 g ha^{-1} result in inhibitory effects. At doses above 36 g ha^{-1}, no significant additional gains in herbicide control are observed.

High IVE values are associated with improved seedling response during crop establishment, promoting faster and more uniform emergence under field conditions (Dan et al. 2012) and reducing exposure to biotic and abiotic factors that could interfere with embryo development (Santos et al. 2018).

Thus, the improper use of glyphosate may negatively affect bean cultivation, causing losses already at crop establishment. These impacts may affect both germination rate and emergence-related indices (IVE and VE). It is observed that intervals longer than 21 days between glyphosate application and sowing have a positive effect, resulting in higher germination rates and better emergence and speed indices for the Urutau bean cultivar.

Effects of Glyphosate on Soil and Germination: Interactions and Variables

Based on the results obtained, it is possible to establish relationships to explain some unusual outcomes observed for the different application times of the glyphosate herbicide on the parameters evaluated in the field experiment. Thus, considering the interaction between glyphosate molecules and soil colloids, with the release of humic compounds, it is possible that these substances positively influenced the treatments, since they possess properties that stimulate the synthesis of plant hormones, such as auxins, as well as enzymes that promote other effects and favor germination, flowering, and shoot growth (Caron et al. 2015).

The experiment conducted in a BOD incubator using a sand substrate allowed for clearer evaluation of glyphosate effects on seed germination, providing representative results regarding residual influence on crop germination. However, from a practical standpoint, this type of dysfunction would be difficult to observe, especially in soils with clayey texture and high organic matter content (Yamada & Castro 2007), as was the case in the field experiment.

In addition, as previously mentioned, microorganisms represent the main pathway for degradation of the glyphosate molecule, along with leaching processes when the compound is present in the soil solution (Prata 2002). Therefore, multiple variables may determine the behavior of glyphosate in the soil–plant system and its influence on bean seed germination.

Final Thoughts

Although subtle, bean germination is affected by residual glyphosate present in soils subjected to pre-sowing desiccation, especially when the interval between management operations is shorter than 21 days. Similarly, a positive correlation between earlier application and initial seedling development was observed, as indicated by IVE. However, with regard to seed vigor, the most favorable desiccation periods were 14 days before sowing and 0 days (spray-and-plant).

Laboratory results suggest interference of glyphosate residue with bean seed germination, with higher values observed when the interval between application and sowing was longer (21 days) or when no glyphosate application was performed (TC). In addition, greater emergence speed was observed in treatments with longer intervals between application and sowing. Furthermore, the Emergence Speed Index was higher in treatments TC and T1.

Thus, further studies are recommended, particularly under field conditions and in years with adequate rainfall levels.

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