Efficacy of glyphosate on David Spurge (*Euphorbia davidii* Subils) control under different levels of P and S

Eficácia do glyphosate no controle David Spurge (*Euphorbia davidii* Subils) em diferentes níveis de P e S

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ABSTRACT

Euphorbia davidii Subils is one of the main weed species present in the central area of Argentina. The objective of this work was to evaluate the efficacy of glyphosate on *E. davidii* control, under different phosphorus and sulfur content in the soil. The aim of this study was to evaluate the influence of the P and S contents in the soil on the efficacy of glyphosate to control *E. davidii*. Two independent experiments were conducted in 2014 and 2015 in a completely randomized design with four replications, using three doses of glyphosate (0, 712, 1068 and 1424 g a.e. ha^{-1}) on plants growing with three levels of P and S (5, 10 and 15 ppm P and SO_4^{-2} respectively). At 5 ppm SO_4^{-2}, the 712 g a.e. ha^{-1} dose of glyphosate showed phytotoxicity ≤50% and evidenced differences with the 1068 and 1424 g a.e. ha^{-1} doses of the herbicide. The shoot dry weight showed only differences between the doses of glyphosate applied at low levels of phosphorus (5 ppm P). Besides, we observed that nutritional deficiency causes a delay in the onset of symptoms of phytotoxicity related to glyphosate. We can conclude that the nutritional status of *E. davidii* plants influenced the efficacy of glyphosate treatments. Finally, we suggest to check and control the nutritional status of agricultural plots and include fertilization practices as part of the information for decision-making within integrated weed management.

Keywords: phosphorus, sulfur, Euphorbiaceae, herbicides, EUDA5

RESUMO

Euphorbia davidii Subils é uma das principais espécies de plantas daninhas presentes na área central da Argentina. O objetivo deste trabalho foi avaliar a eficácia do glyphosate no controle de E. davidii, sob diferentes teores de fósforo e enxofre no solo. O objetivo deste estudo foi avaliar a influência dos teores de P e S no solo sobre a eficácia do glyphosate no controle de E. davidii. Dois experimentos independentes foram conduzidos em 2014 e 2015 em delineamento inteiramente casualizado com quatro repetições, utilizando três doses de glyphosate (0, 712, 1068 e 1424 g ae ha-1) em plantas cultivadas com três níveis de P e S (5, 10 e 15 ppm de P e SO4-2 respectivamente). A 5 ppm de SO4-2, os 712 g e.a. A dose ha-1 de glyphosate apresentou fitotoxicidade ≤50% e evidenciou diferenças com as doses de 1068 e 1424 g e.a. ha-1 do herbicida. A massa seca da parte aérea apresentou diferenças apenas entre as doses de glyphosate aplicadas em níveis baixos de fósforo (5 ppm P). Além disso, observamos que a deficiência nutricional causa retardo no aparecimento dos sintomas de fitotoxicidade relacionados ao glyphosate. Podemos concluir que o estado nutricional das plantas de E. davidii influenciou a eficácia dos tratamentos com glyphosate. Por fim, sugerimos verificar e controlar o estado nutricional das parcelas agrícolas e incluir as práticas de adubação como parte das informações para a tomada de decisões no manejo integrado de plantas daninhas.

Palavras-chave: fósforo, enxofre, Euphorbiaceae, herbicidas, EUDA5

1 INTRODUCTION

Euphorbia davidii Subils (Euphorbiaceae) is a weed species with an annual spring-summer cycle, native to countries of the northern hemisphere of America (USA, Canada and Mexico), recently introduced in Australia, Europe, and South America (Argentina) (Geltman, 2012). In the Central and Southeast part of Buenos Aires province, Argentina, *E. davidii* presents a high distribution, density and abundance in soybean cropping systems, resulting in a significant crop yield reduction. In Argentina, in the center and southeast of Buenos Aires province, this weed causes significant yield reductions in soybean crops and, in the past two decades, has shown an increase in its constancy, density and coverage (Núñez Fre et al., 2014).

Little is known about management strategies to control this weed, but most are based on the use of glyphosate (N- phosphonomethyl glycine), the herbicide most widely used worldwide. In Argentina, however, Juan et al. (2011) reported that control of *E. davidii* in soybean crops by using glyphosate is becoming increasingly difficult. The weed phenological stage has a direct influence on the control efficacy obtained with glyphosate. From the branching stage, particularly at flowering it becomes harder to achieve controls over 75% at label doses of glyphosate applied (Juan et al., 2011). Since the introduction of glyphosate-resistant crops, the intensity of use of this herbicide in agriculture has markedly increased. Although initially allowed control of weeds in such crops, the recurrent use of glyphosate has led to the emergence of numerous cases of resistance due to selection pressure (Heap and Duke, 2018).

Glyphosate mode of action consists of the inhibition of the enzyme 5-*enolpyruvylshikimate-3-phosphate synthase* (EPSPs; EC 2.5.1.19) (Amrhein et al.,1980). This inhibition leads to the blockage of the *shikimate* pathway, which in turn causes a reduction in the synthesis of aromatic amino acids and proteins, a reduction in growth, and premature cell death (Duke, 1988).

On the other hand, the efficacy of glyphosate may be affected by several factors, including the weather conditions at the time of application (temperature, subsequent rains, etc), the quality of water (pH, hardness, presence of clay or organic matter, etc) and the phenological and physiological stage of the weed (Menéndez et al., 1999). In addition, since glyphosate is a systemic herbicide that is translocated within the plants in the same way as photoassimilates, any factor that limits its absorption or translocation within the plant can decrease its effect on weeds (Menéndez et al., 1999).

Although glyphosate is applied at the post-emergence of weeds, its activity is also influenced by the characteristics and nutrient content of the soil (Ncedana, 2011). In the center and southeast of Buenos Aires province, the soils are characterized by deficiencies in mineral nutrients, mainly nitrogen (N), phosphorus (P) and more recently sulfur (S), which affect agricultural production.

Phosphorus (P) is an important macronutrient, which makes up about 0.2% of the dry weight of plants, and is essential for all living organisms because it is part of many sugar-phosphates involved in photosynthesis, respiration and other metabolic processes. It is also part of nucleic acids (such as RNA and DNA) and membrane phospholipids, and involved in energy metabolism, due to its presence in molecules of ATP, ADP, AMP and pyrophosphate (Salisbury and Ross, 1991). In addition, P is part of phosphoenolpyruvate, a metabolic intermediate in the synthesis of aromatic amino acids, involved in the action of glyphosate. P-deficient plants also have delayed growth and a low shoot/root dry weight ratio (Mengel and Kirkby, 2000). P-deficient herbaceous plants also accumulate anthocyanins, which produces a reddish coloration at the base of the stems. Under P-deficient conditions, inorganic P is transported from the shoot tissues to the roots via the phloem (Mimura, 1999). In response to the low availability of P, plants exhibit several physiological, morphological and architectural changes in the roots (Lynch, 1997), which include the generation of a larger number and greater elongation of root hairs.

Sulfur is also essential for plant growth. This macronutrient is found in the soil in inorganic and organic forms. In most soils, organic S provides the main S reservoir (Reisenauer et al., 1973). Sulfur is found mainly in an oxidized state in the form of sulfate (SO_4^{-2}), which is susceptible to leaching. The availability of sulfur in terrestrial ecosystems is very variable, from very low in sandy soils to extremely abundant in soils from tidal zones (Stevenson and Cole, 1999). S deficiency in plants causes an imbalance at the physiological level, which is reflected at the agronomic level (Malhi et al., 2005). Plants suffering from S deficiency show decreases in the growth rate, foliar expansion rate, and photosynthesis (Marschner, 2012), all of which lead to a decrease in the production and migration of photo-assimilates within the plant.

All this indicates that a low availability of nutrients in the soil could be a limiting factor in the efficacy of glyphosate. Some studies have shown that the translocation of glyphosate within plants is reduced by the lower level of photosynthesis caused by N stress. In oat (*Avena sativa* L.) crops, systemic herbicides such as glyphosate and fluazifop show less phytotoxicity in soils with low N availability than in soils with high N availability (Dickson et al., 1990). However, this response is not always observed, because no significant differences in the control of *Ambrosia artemisiifolia* between soils with low and high N availability were observed, suggesting that the response to the glyphosate-N interaction in the soil is species-dependent (Mithila et al., 2008).

Similarly to that documented for N, the effects of herbicides seem to be related to the P content. Some researchers have found that P availability in the soil is a factor influencing the phytotoxicity of several herbicides. Adams and Russell (1965), for example, found that the phytotoxicity of the herbicide simazine in soybean is higher in soils with high P levels than in those with low levels. On the other hand, Upchurch et al. (1963) studied the influence of P content on the phytotoxicity of several herbicides and found significant evidence of increased herbicide phytotoxicity in soils with high P levels. In addition, according to Rahman (1978), the initial phytotoxicity of alachlor in a sensitive species such as Foxtail millet (*Setaria italica* (L.) P. Beauv.) decreases as P levels increase. Wilson and Stewart (1973), meanwhile, found interaction between the P levels in the soil and the dose of trifluralin applied in the growth of tomato plant roots. However, these records on the interactions between herbicide phytotoxicity and the P level in the soil refer to soil-applied herbicides, and, to our knowledge there are no studies of the interactions with post-emergence foliar-absorbed herbicides, such as glyphosate. In addition, we found no studies regarding the interaction between the content of S in the soil and the action of herbicides.

Thus, the aim of this study was to evaluate the influence of the P and S contents in the soil on the efficacy of glyphosate to control *E. davidii*. We hypothesize that the deficiency of these nutrients makes it difficult to control with glyphosate.

2 MATERIALS AND METHODS

Two independent greenhouse experiments were carried out, one with each nutrient (P and S), at the Facultad de Agronomía de la Universidad Nacional del Centro de la Provincia de Buenos Aires, Argentina (36°46'01.2"S 59°52'54.1"W).

The soil used for the two experiments was obtained from the sandy-loam A horizon, from a farm located in Bolívar, Province of Buenos Aires (36-33'26.0"S 61-02'02.4"W). Soil samples were extracted from two plots, and chemical analysis of the three main nutrients (N, P, and S), organic matter, and pH were performed before the experiments (Table 1).

For the P test, sample "A" was used, which contained a P level of 5 ppm as stated by chemical analysis. To establish increasing levels of this nutrient, KH_2PO_4 there was added as a source of P, and treatments were defined as low level of P (5 ppm), intermediate level of P (10 ppm), and high P (15 ppm). The N level was homogenized at 40 ppm NO_3^- using KNO_3. These nutrients were sprayed over the soil (sample "A") with a solution containing the calculated amount of KH_2PO_4 and KNO_3 and then mixed it to obtain a homogeneous concentration of nutrients.

For the test with S, it was used sample "B", which according to the chemical analysis had 5 ppm $SO4^{-2}$ and from this, the treatments were defined as low level of S (5 ppm SO_4^{-2}), intermediate level of S (10 ppm SO_4^{-2}), and high level of S (15 ppm SO_4^{-2}), adjusting the amount by adding $SO_4(NH4)_2$. The level of P was adjusted to 15 ppm using KH_2PO_4 and the N content was adjusted to 40 ppm of NO_3^- adding KNO_3 as a source of N, taking into account the amount of N provided by the source of S, which was $SO_4(NH_4)_2$. As in the phosphorus experiment, the nutrients were incorporated by spraying an aqueous solution on the soil samples and mixing it.

Table 1. Results of the analyses of the samples extracted from the soil, prior to their use for the phosphorus and sulfur experiments.

	OM (%)	pH	Nitrates (NO_3^- ppm)	Bray phosphorus (ppm)	Sulfur (SO_4^{-2} ppm)
Sample A	4.6	6.4	31.3	5	8
Sample B	3.8	6.9	14.1	3	5

After adjusting the level of P and S, the soil samples were distributed in one-liter pots as substrate and 30 seeds of *E. davidii* were sown in each pot. The seeds used for these tests were obtained from mature plants that grew under natural conditions in the district of Olavarría, Province of Buenos Aires.

The experiments were placed in a greenhouse under semi-controlled conditions of temperature and humidity, with periodic watering, so that the plants grow without water limitation.

Seven days after emergence, the pots were thinned leaving five seedlings per each experimental unit.

Before the plants began to branch, a soluble concentrated solution of 40.5% ammonium glyphosate salt was applied, 356 g acid equivalent (a.e.) L^{-1}, in doses of 0, 2, 3 and 4 L ha^{-1}, which corresponded to 0, 712, 1068 and 1424 g a.e. ha^{-1}.

For this purpose, the plants were treated in a spray chamber equipped with CO_2 equipment, with a constant 3-bar pressure, through a Teejet flatfan spray nozzle (ST8001 – Spay angle 80º, nozzle discharge 0.1-gal min^{-1}), spraying a flow equivalent to 130 L ha^{-1}.

The efficacy of each dose of glyphosate was evaluated at 7, 14, and 21 days after application (DAA), according to a visual evaluation scale, where 0% corresponded to the absence of symptoms and 100% to the total death of weeds.

At 28 DAA, the plants were cut at ground level and placed in the drying chamber at 60 °C for 3 days to record the shoot dry weight. In the S experiment, phytotoxicity was evaluated up to 28 DAA, since some significant visual changes were observed at the time of cutting.

Phosphorus and sulfur experiments had four replications per treatment, they were carried out twice (2014 and 2015) and the statistical analysis grouped information from both experiments. Normality tests and data analysis showed that no transformations were necessary to be performed. All data obtained were analyzed by ANOVA, and the means were compared by Tukey test ($p \leq 0.05$), using the statistical software InfoStat v. 2012 (Universidad Nacional de Córdoba, Argentina).

3 RESULTS AND DISCUSSION

In both experiments, statistical analyses showed interaction between the nutrient content in the soil and the glyphosate doses. Thus, the results for each nutrient level were analyzed separately.

3.1 INFLUENCE OF P CONTENT IN THE SOIL ON THE EFFICACY OF GLYPHOSATE TO CONTROL EUPHORBIA DAVIDII SUBILS

As expected, when analyzing phytotoxicity based on the visual scale proposed, we observed a tendency to higher phytotoxicity as the dose of glyphosate increased. In addition, we observed differences in the phytotoxicity of the same treatment according to the date of evaluation, with maximum phytotoxicity, in most cases, at 21 DAA (Table 2).

At a 5 ppm P level, at 7 DAA all treatments with glyphosate (even the highest dose, 1424 g a.e. ha^{-1}) demonstrated low percentages of phytotoxicity (less than 30%). At 14 DAA, 712 and 1068 g ae ha^{-1} phytotoxicity increased, reaching approximately 60% (Table 2). However, these values were not significantly different from those observed with the highest dose, which recorded the total mortality of the plants (100% phytotoxicity). In the last evaluation at 21 DAA, 712 and 1068 g ae ha^{-1} showed an increase in phytotoxicity, reaching values around 75%.

At the 10 ppm P level (Table 2), the results were different from those observed at the 5 ppm P level. At the 7 DAA, statistically significant differences were observed between the highest dose (which reached a phytotoxicity close to 50%) and the lowest dose (712 g ae ha^{-1}), whose phytotoxicity value was below 20%. At 14 DAA, phytotoxicity increased significantly in all treatments, reaching values of approximately 80, 90, and 100% for doses 712, 1068 and 1424 g ae ha^{-1} respectively. At 21 DAA, phytotoxicity was equal to or greater than 90% in all treatments, with no significant differences between them.

In the soil with 15 ppm of P (Table 2), significant differences were detected in the first evaluation (7 DAA). The 1424 g ae ha^{-1} dose showed phytotoxicity of approximately 45%, differing from 712 g ae ha^{-1}, which presented phytotoxicity values lower than 20%. At 1068 g ae ha^{-1} dose phytotoxicity of 35% was observed, without significant difference with the other doses evaluated. At 14 DAA, phytotoxicity increased in all treatments, reaching 100% with 1068 and 1424 g ae ha^{-1}. However, these doses did not show differences with the lowest dose, which showed phytotoxicity values of 75%. At 21 DAA, the phytotoxicity values found were similar to those observed at 14 DAA.

Table 2. Evolution of phytotoxicity at the different times of evaluation, at glyphosate doses of 712 (D1), 1068 (D2) and 1424 (D3) g a.e. ha^{-1}, for different phosphorus levels. Comparisons are only valid within each date and nutrient level. Mean comparisons (minimum significant difference; MSD) were established according to Tukey's test ($p<0.05$).

P levels in the soil	Phytotoxicity (%)			
	Dose	Days after application		
	g a.e. ha^{-1}	7	14	21
Low P	712	13.8	63.3	76.0
	1,068	14.4	59.4	77.0
	1,424	24.9	100	100
MSD $p\leq 0.05$		17.2	37.1	43.1
Intermediate P	712	17.9	78.8	90.0
	1,068	23.8	88.7	100
	1,424	46.3	100	100
MSD $p\leq 0.05$		16.1	22.3	18.4
High P	712	18.0	74.4	74.0
	1,068	35.2	100	100
	1,424	45.6	100	100
MSD $p\leq 0.05$		20.1	23.5	33.9

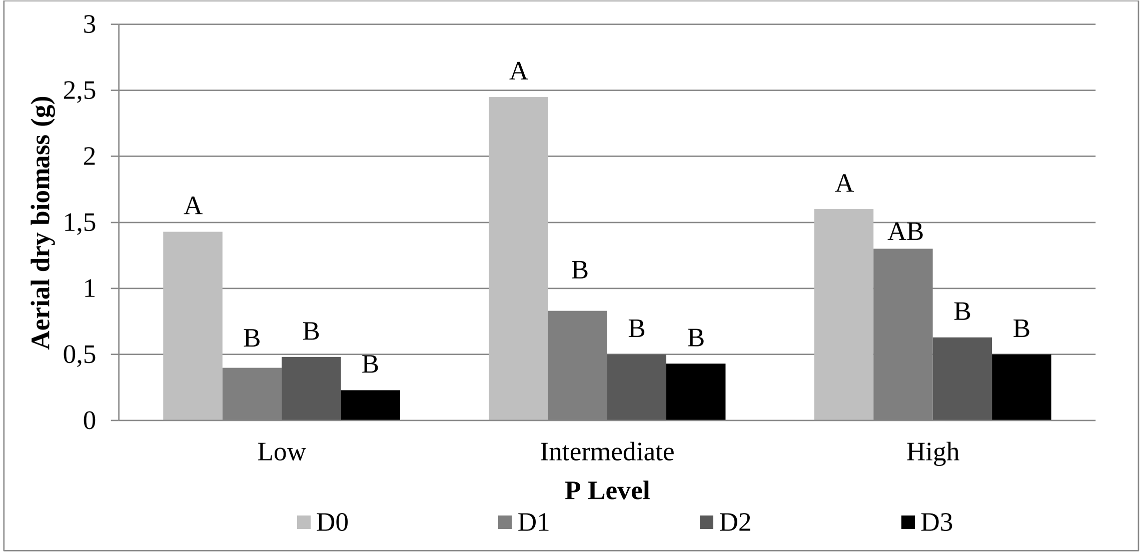
Although at the different P levels evaluated, all the treatments reached similar phytotoxicity values at the end of the experiments, a difference in the rate of appearance of symptoms was observed. At low P levels (5 ppm P), all glyphosate doses reached 50% phytotoxicity in 14 days, while at intermediate and high P levels (10 and 15 ppm P), the highest glyphosate dose reached a phytotoxicity value close to 50% at 7 DAA, while the other two doses took 14 days to make an equivalent result. Possibly, the increased availability of P to weeds would allow the herbicide to move more quickly to the site of action, resulting in the earlier development of symptoms.

Regarding the shoot dry weight, in general, it was observed a decrease in its accumulation as the glyphosate dose increased in all the evaluated P levels (Figure 1). At the high level of P (15 ppm P), the lowest dose of glyphosate (712 g ae ha^{-1}) caused a decrease in shoot dry weight of approximately 50%, while 1068 and 1424 g ae ha^{-1} caused biomass reduction larger than 65% and did not differ from each other.

At the intermediate level of P (10 ppm P), all the doses differed from the control treatment, which caused reductions in the accumulation of dry biomass of more than 70%. In this case, no significant differences were found between the three glyphosate doses evaluated.

At the 5 ppm level of P, 712 and 1068 g ae ha^{-1}, the accumulation of biomass was 30% concerning the control, detecting significant differences with it. On the other hand, 1424 g ae ha^{-1} dose presented significant differences with 712 and 1068 g ae ha^{-1}, causing a reduction in the accumulation of dry biomass of more than 80%.

Figure 1. Aerial dry biomass of *E. davidii* determined at 28 DAA on plants growing in a soil with different levels of phosphorus, treated with glyphosate doses of 712 (D1), 1068 (D2), 1424 (D3) g a.e. ha^{-1} and control without the herbicide (D0). Comparisons are only valid within each phosphorus level. Different letters indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).



These results show an influence of the nutritional level of P in the control of this weed with glyphosate. If we consider the 70% reduction in shoot dry weight compared to the control as a threshold for comparison, clearly this value is only achieved with the highest dose (1424 g ae ha^{-1}) at the high level of P (15 ppm P), with 1068 and 1424 g ae ha^{-1} in low P level (5 ppm P), and with all doses at the intermediate level of P (10 ppm P).

3.2 INFLUENCE OF THE SULFUR CONTENT IN THE SOIL ON THE EFFICACY OF GLYPHOSATE TO CONTROL EUPHORBIA DAVIDII

In general, higher levels of phytotoxicity were observed with increase in dose of glyphosate applied and in the successive evaluations at different moments after application.

In the treatments with low S (5 ppm SO_4^{-2}), at 7 DAA was not possible to establish significant differences between the applied glyphosate doses: 1424 g ae ha^{-1} achieved at 40% phytotoxicity, while the 712 and 1068 g ae ha^{-1}doses achieved slight phytotoxicity (about 20 %) (Table 3). In the next evaluations, a linear increase in phytotoxicity with 1068 and 1424 g ae ha^{-1} was observed, reaching phytotoxicity values of 67 and 85% respectively at 21 DAA. The lowest dose of glyphosate (712 g ae ha^{-1}) showed a moderate increase in phytotoxicity, getting to 40% on the same evaluation date, but did not differ from the rest of the treatments. However, in the last evaluation at 28 DAA, the lowest dose showed only 50% phytotoxicity, while 1068 and 1424 g ae ha^{-1} exceeded 90% phytotoxicity.

At the intermediate S level (10 ppm SO_4^{-2}), there were notable differences at the 7 DAA (Table 3). The 1424 g ae ha^{-1} dose presented the highest phytotoxicity (50%) showing significant differences with 712 g ae ha^{-1} whose phytotoxicity was approximately 20%. The intermediate dose of glyphosate (1068 g ae ha^{-1}) showed 30% phytotoxicity and had no significant difference with 712 and 1424 g ae ha^{-1}. At 21 DAA, 1424 g ae ha^{-1} showed phytotoxicity close to 80%, 712 g ae ha^{-1} evidenced the lowest phytotoxicity (45%) and 1068 g ae ha^{-1} dose had an intermediate result close to 60%, which had no significant difference with the other glyphosates treatments. In the last evaluation (28 DAA), 1068 and 1424 g ae ha^{-1} showed phytotoxicity values higher than 80%, while 712 g ae ha^{-1}only reached 70%. However, there were no significant differences between the three doses at this point in the evaluation.

At the high level of S (15 ppm SO_4^{-2}) (Table 3), we also detected significant differences at the 7 DAA between 712 g ae ha^{-1}, which presented phytotoxicity lower than 20%, and 1068 and 1424 g ae ha^{-1}, which presented phytotoxicity values higher than 40%. The symptoms continued to evolve and, at 21 DAA, 1068 and 1424 g ae ha^{-1} showed phytotoxicity values higher than 80%. At the same time of evaluation, 712 g ae ha^{-1} reached only 50% phytotoxicity with significant differences for the other two doses.

At the end of trials, glyphosate doses 1068 and 1424 g ae ha^{-1} showed phytotoxicity values close to 100%, not differing from each other, but with a clear difference with 712 g ae ha^{-1} that manifested 70% phytotoxicity.

Table 3. Evolution of phytotoxicity for different times of evaluation, at glyphosate doses of 712 (D1), 1068 (D2) and 1424 (D3) g a.e. ha^{-1}, for different sulfur levels. Comparisons are only valid within each date and nutrient level. Mean comparisons (minimum significant difference; MSD) were established according to Tukey's test ($p<0.05$).

Levels of S in the soil	Phytotoxicity (%)				
	Dose	Days after application			
	g e.a. ha^{-1}	7	14	21	28
Low S	712	20.0	33.3	40.0	50.0
	1,068	22.5	47.5	67.5	92.5
	1,424	42.5	65.0	85.0	100
MSD $p\leq 0.05$		27.9	36.6	39.8	15.7
Intermediate S	712	22.5	42.5	45.0	70.0
	1,068	30.0	50.0	57.5	82.5
	1,424	50.0	65.0	77.5	92.5
MSD $p\leq 0.05$		21.8	23.6	22.9	20.9
High S	712	17.5	47.5	50.0	70.0
	1,068	52.5	70.0	82.5	97.5
	1,424	45.0	57.5	82.5	97.5
MSD $p\leq 0.05$		13.5	9.9	16.4	14.6

Unlike the phosphorus experiment, in the last evaluation of phytotoxicity (at 28 DAA), at low and high levels of S (5 and 15 ppm SO_4^{-2}), the lowest dose of glyphosate showed a lower level of phytotoxicity that was significantly different from the other treatments.

Taking into account the rate of appearance of symptoms, at the highest dose used, phytotoxicity reached approximately 50% at 7 DAA at all S levels. The intermediate dose (1068 g a.e. ha^{-1}) required 14 days to reach the same phytotoxicity at the low and intermediate S levels (5 and 10 ppm SO_4^{-2}), and 7 days to reach the same phytotoxicity at the high S level (15 ppm SO_4^{-2}). Finally, the most notable differences were observed at the lowest dose evaluated, which required 28, 21 and 14 days to reach 50% phytotoxicity at the low, intermediate and high S levels respectively.

These results indicate that the level of S in the soil has an influence, although limited, on the post-emergence control of this weed with glyphosate. With the use of low glyphosate doses, the influence of the nutritional status is more clearly observed, where inadequate nutrient availability can affect different metabolic processes within the plant, and possibly prevent the herbicide from reaching the site of action.

With respect to shoot dry weight, unlike that observed with the level of P (Figure 1), no differential behavior was observed between the S levels assessed (Figure 2).

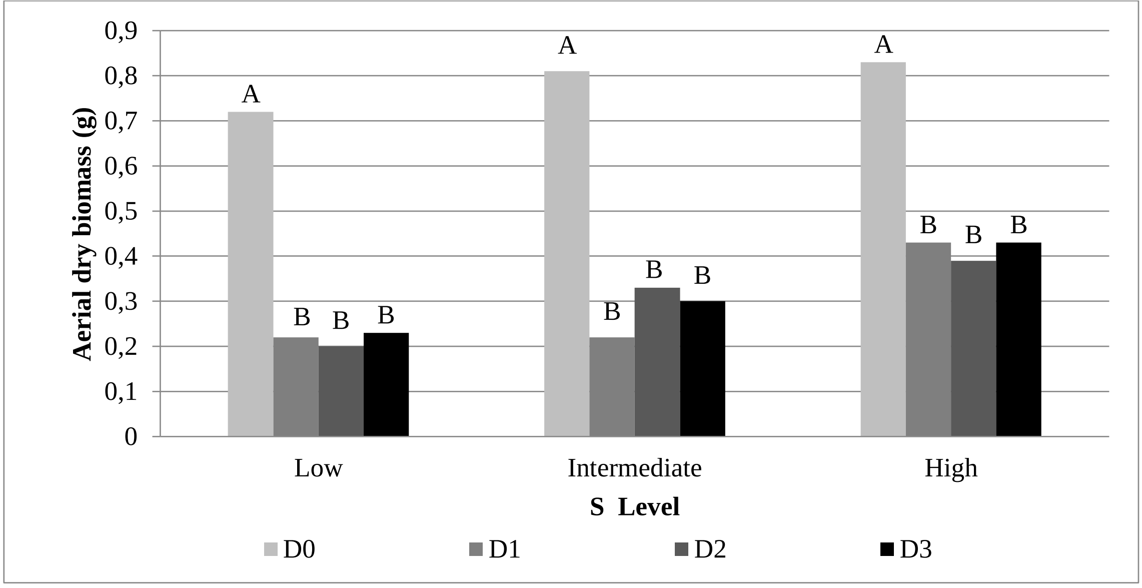
No significant differences were observed between the three glyphosate doses used at any of the S levels assessed, resulting in reductions in dry biomass accumulation of 70, 65 and 50% compared to the control for the low, intermediate and high S levels respectively (5, 10 and 15 ppm SO_4^{-2}), with no significant differences between them. This lower inhibition of the relative growth as the S level increased could have been caused by increased growth and shoot dry weight accumulation prior to the application of the herbicide at intermediate and high S levels (10 and 15 ppm SO_4^{-2}), showing effects of growth retardation due to nutritional stress at low S levels.

As mentioned above, since glyphosate is a systemic herbicide that is transported within plants in the same way as photoassimilates, any factor that limits its absorption or transport within the plant can decrease its effect on weeds (Menéndez et al., 1999).

Some researchers have mentioned the interaction between the P levels and soil herbicides (Upchurch et al., 1963; Adams and Russell, 1965), but there is no documented information on the interaction between the P levels and post-emergence herbicides.

Mineral nutrient deficiencies can affect the transport and cycling of molecules within the plant (Marschner, 2012), a fact that is accompanied by changes in the source-sink relationship between different organs, functioning as adaptive strategies that allow modifying the stem-root relationship (Mengel and Kirkby, 2000).

Figure 2. Aerial dry biomass of *E. davidii* determined at 28 DAA on plants growing in soil with different sulfur levels, treated with glyphosate doses of 712 (D1), 1068 (D2), 1424 (D3) g a.e. ha^{-1} and control without the herbicide (D0). Comparisons are only valid within each sulfur level. Different letters indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).



P deficiencies significantly affect the transport of simple sugars as sucrose (Hammond and White, 2008), as well as of amino acids, in interaction with the availability of other nutrients such as nitrogen (Criado et al., 2017). Similarly, S deficiencies generate metabolic changes within the plant, which affect the transport of this nutrient (Hawkesford, 2000) and modify the absorption and transport of other nutrients (Alhendawi et al., 2005). If the phloem transport under these conditions is affected, the transport of post-emergence systemic herbicides, such as glyphosate, is also likely affected.

As described, in the present study, we observed differences in the rate of appearance of symptoms due to the phytotoxic effect of glyphosate between the different levels of nutrients evaluated. Although the nutritional status regarding both P and S may affect the absorption or transport of glyphosate within the plant, these effects seemed to be more noticeable under S deficiency, when considering phytotoxicity as the evaluation parameter. However, more evaluations are needed to confirm these results.

In contrast to the differences found in the rate of appearance of symptoms, we found no significant differences in the final phytotoxicity induced by the herbicide doses evaluated at any of the P levels evaluated. In the case of S, while the lowest glyphosate dose (712 g ae ha^{-1}) led to values statistically lower than those achieved by the other doses at low and high S levels (5 and 15 ppm SO_4^{-2}) (Table 3), this was not evident in the determinations of shoot dry weight (Figure 2). In this case, there may be an even more complex interaction than in the case of P. On the one hand, there may be less transport within the plant, due to the nutritional deficiency, which prevents the herbicide from reaching the site of action, but it is also important to consider the role of S as part of the mechanisms of detoxification of xenobiotics. In addition to hydrolysis reactions, one of the main inactivation pathways of the biological activity of the herbicide within the plant is conjugation, which consists of the binding of the herbicide to different metabolites and plant nutrients such as sucrose, glucuronic acid, sulfate, and especially glutathione (Hirase and Molin, 2003).

Kuzuhara et al. (2000) showed that S deficiency could change the sulfate levels within the plant quickly, whereas glutathione levels remain stable in limited deficiencies (less than 10 days). It is possible that, in longer stress situations such as in the case of this study, the plant will deplete its glutathione and/or sulfate reserves, a fact that would limit its ability to detoxify herbicides.

4 CONCLUSIONS

The results obtained in the present work show that the nutritional level of the soil in which *E. davidii* plants grew, influenced the efficacy of glyphosate treatments to control this weed.

At low and high sulfur levels (5 and 15 ppm SO_4^{-2}), data showed that the lowest dose of glyphosate (712 g ae ha^{-1}) showed a low level of phytotoxicity that presented a significant difference with the intermediate and high amounts of the herbicide. The shoot dry weight showed notable differences between the doses of glyphosate applied only at low levels of phosphorus (5 ppm P). Besides, differences were observed in the rate of appearance of symptoms at different levels of both nutrients, observing that nutritional deficiency causes a delay in the onset of symptoms.

We can conclude that *E. davidii* plants in an adequate growth and with adequate nutrient intake responded better to herbicide treatments within the evaluated dose range, evidenced by the rate of appearance of symptoms and the control result. Therefore, we can affirm that the nutrient deficiency present in the soils of the center of the province of Buenos Aires, Argentina, could partly explain the unpredictable results of the control of this weed observed in the agricultural production plots of the region.

It is suggested to control the nutritional level of agricultural plots, and include fertilization practices as part of the information for decision-making within integrated weed management.

AUTHOR CONTRIBUTIONS

All the authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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