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Glyphosate hormesis variation in common bean due to nitrogen coverage

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Abstract. In order to evaluate the influence of different nitrogen rates on the hormetic effect of glyphosate on common bean crop, proposed to study the combination of nitrogen fertilization in succession to low glyphosate doses. The study was conducted under field conditions at the experimental farm of the State University of São Paulo-UNESP, Ilha Solteira campus, located in the city of Selvíria-MS, Brazil. The experimental design was a randomized block with split plots arranged in four blocks. The plots were composed of four glyphosate doses (0, 7.2, 14.4 and 21.6 g of acid equivalent (AE) ha⁻¹) and the subplots were composed of five nitrogen doses (0, 15, 30, 45 and 60 kg ha⁻¹), with urea as the source. Bean growth was stimulated with a maximum point in the 11 g AE ha⁻¹ sub-dose of glyphosate, however, only in the absence of nitrogen under cover. Leaf N content was higher at the estimated 12 and 11 g AE ha⁻¹ glyphosate doses with 30 and 45 kg ha⁻¹ nitrogen. Hormetic effect on yield was obtained with increments of up to 500 kg ha⁻¹ or 21% at the estimated 10 g AE ha⁻¹ low glyphosate dose. Cooking time was affected by low glyphosate doses, depending on the amount of nitrogen. It was possible to prove the influence of nitrogen on glyphosate hormesis in common bean for dry matter mass, leaf N content, grain yield and cooking time.

Keywords: *Phaseolus vulgaris* L., nitrogen fertilization, hormetic effect, herbicide, interference.

Introduction

The Common Bean stand out as a fundamental component in the diet of Brazilians. Together with rice, they are able to meet much of the daily protein demand and mainly provide two essential amino acids to the body such as lysine and methionine. Moreover, in Brazil, beans can be grown in up to three harvests, the first being called the “water” crop, the second “dry” crop and the third “winter” crop.

In the field, several factors directly and indirectly affect the quantity and quality of the harvested product. Among them, plant mineral nutrition is of fundamental importance.

Nitrogen (N) is the nutrient extracted in largest amounts by the common bean crop, which exports on average 35 kg N per ton of grains produced (Ambrosano et al., 1997). However, more recent research indicates an average export of 25 kg N per ton of grain (Soratto et al., 2013; Leal et al., 2019).

These N fertilizers are expensive, burn petroleum during their production, and their

excessive use may contaminate water courses. Petroleum is a finite resource; its combustion pollutes atmosphere and its extraction affects marine life (Leal et al., 2019). Therefore, numerous researches have been conducted to reduce the applications of this nutrient due to the high cost that this operation brings to the final product.

One technique still little used in this regard is the phenomenon of hormesis. It is the use of a toxic substance in low doses or subdoses to obtain positive results in characteristics such as plant growth and even productivity.

In seeking to achieve high productivity with good profitability, the use of new technologies is indispensable. Thus, several studies have shown hormetic effect in cultivated plants (Silva et al., 2013a).

According to Hashmi et al. (2014), beneficial plant growth responses to low doses of toxic compounds have been observed by weed researchers for several decades. The authors also point out that plants are sessile organisms and therefore cannot physically escape from an adverse

environment. However, under harsh environmental conditions, they have the ability to allocate their resources to optimize their growth.

One of the herbicide molecules that has shown the most positive results for plant hormesis is glyphosate (Belz; Duke, 2014). Glyphosate (N-phosphonomethyl-glycine) is the world's leading herbicide (Service, 2007; Rabello; Monnerat; Vasconcelos Júnior, 2014). Its mechanism of action is based on the inhibition of the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPs). This enzyme is critical in the shikimic acid pathway and its inhibition results in reduced production of aromatic amino acids (phenylalanine, tyrosine and tryptophan), impairing protein synthesis and photosynthetic process (Rabello; Monnerat; Vasconcelos Júnior, 2014; Silva et al., 2016a).

Silva et al. (2013a) concluded that it is possible to obtain a higher gain margin by using glyphosate subdoses for Juriti and Pérola common bean cultivars, as well as, other research has shown increased in productivity of bean crop (Silva et al., 2012; Silva et al., 2016a; Silva et al., 2016b) and the increase of the biomass in soybean (Velini et al., 2008), curauá branco (Maciel et al., 2009), *Brachiaria brizanta* cv. Marandu (Nascentes et al., 2015) and sugarcane and eucalyptus (Nascentes et al., 2017). In addition, another herbicidal molecule has shown results, such as 2,4-D in soybean (Silva et al., 2019). On the other hand, Ries et al. (1967) and Pulver and Ries (1973) developed considerable evidence that low doses of the triazine chemical group simazine herbicide could improve nitrogen metabolism in some cultures, but this information has not been explored. Which makes the relationship of the hormetic effect with plant nutrition

and nitrogen fertilization a wide range to be explored and that can bring benefits to agricultural crops.

In this context, the present study aimed to evaluate the effects of nitrogen application in coverage in succession to the application of glyphosate subdoses on the development, nutrition, productivity and technological quality of common bean.

Methods

Plant materials

The common bean cultivar utilized was IPR 139 (white Juriti), developed by the Agronomic Institute of Paraná of the Carioca Group, with indeterminate growth habit (Type II), shrubby, erect plant size and little branched stem.

Experimental conditions and soil conditions

The study was conducted in the experimental area belonging to the Engineering College of Ilha Solteira - UNESP, located in Selvíria - MS, from May to August 2014, with geographic coordinates of 51° 24 '1.12" W and 20° 20' 51, 27" S and average elevation of 344 meters.

The climate type is Aw, according to Köppen (2004), characterized as tropical humid with rainy summer season and dry in winter.

The soil was classified according to Santos et al. (2013), as clayey Red Oxisol. The experiment was installed in an area previously occupied by maize crop under no-tillage system. The chemical characteristics of the soil were determined before the experiment, following the methodology proposed by Raij and Quaggio (1983). The results are presented in Table 1:

Table 1. Chemical analysis of the soil of the experimental area, Selvíria-MS, Brazil.

Depth (m)	pH	P-resin	K	Ca	Mg	Al	H + Al	CTC	V	OM
	CaCl ₂	mg dm ⁻³			mmolc dm ⁻³				%	g dm ⁻³
0,00 – 0,05	6,0	34	1,9	41	23	0	17	82,9	79	21
0,05 – 0,10	5,6	38	1,3	30	16	0	22	69,3	68	16
0,10 – 0,20	4,6	31	1,3	22	13	0	22	58,3	62	15

Source: Data obtained from the soil fertility laboratory of FEIS - UNESP.

Experimental design

The experimental was setup in a randomized block with split plots arranged in four blocks. The plots were composed of four glyphosate doses (0, 7.2, 14.4 and 21.6 g of acid equivalent (AE) ha⁻¹) and the subplots were composed of five nitrogen doses (0, 15, 30, 45 and 60 kg ha⁻¹), with urea as the source. Plots consisted of 14 lines measuring 12 meters long and the subplots consisted of 6 lines measuring 5 meters long, where the 3 central lines were used as the working area, disregarding 0.5 m at the end of each row.

Plant conduction

Basic chemical fertilization in the sowing furrows was performed while taking into account the chemical characteristics of the soil and the recommendations of Ambrosano et al. (1997). For this reason, 210 kg ha⁻¹ was applied of the formula 8-28-16. Seeds were sown on may, 15, 2014, using the spacing of 0.45 m between rows, and seeds needed to produce 12-13 plants m⁻¹ after emergence. Before sowing, the seeds were treated with the insecticide fipronil (50 g 100 kg⁻¹) and fungicide carboxin + tiran (50 + 50 g 100 kg⁻¹).

Emergence of the plants occurred seven days after sowing (DAS) and harvest at 96 days after emergence (DAE). Weed control was performed at 16 DAE with the herbicide bentazone

(720 g ha⁻¹). The remaining cultural practices were those usually recommended for the common bean crop in the region.

Water supply

Irrigation was performed with a 13 mm water depth during the most demanding crop periods (R₅ to R₇) by a central pivot sprinkler irrigation system. Water replacement was performed when accumulated crop evapotranspiration (ET_c) reached values close to the pre-established available ground water (ADS). Water evaporation (ECA) was obtained daily from the class A tank installed at the Meteorological Station 500 m from the experimental area. The coefficient of the class A (K_p) tank used was that proposed by Doorenbos and Pruitt (1976).

Glyphosate treatments

The application of low glyphosate doses (Roundup Original® - 360 g L⁻¹) was carried out as a foliar spray, with the aid of a CO₂ pressurized backpack sprayer and a spray bar with five spray nozzles, model TXA 8002 VK, maintaining a constant pressure of 3 kgf pol⁻² and spray volume of 160 L ha⁻¹, with a displacement of 1 ms⁻¹ at 0.5 meter in height in relation to the target. This application was performed at 27 days after emergence (DAE) of the V₄₋₅ stage plants or with the fifth fully formed leaf. To ensure the quality of the operation, the application of glyphosate was performed in periods of milder temperatures (late afternoon) and low wind interference.

Nitrogen treatments

Nitrogen fertilization was carried out in the rows using urea (45% N), 30 DAE, when plants reached the V4-6 stadium, or sixth fully formed leaf.

Variables measured

The plant population was evaluated by counting the plants in 2 rows of the plot useful area at the end of the crop development and the data were transformed into plants m⁻¹. At 7 and 14 DAT, 10 random plants were collected in the area of each plot to determine plant dry matter mass. The samples were taken to the laboratory, packed in properly identified paper bags and placed for drying in a forced air circulation oven at 60 – 70 °C, until reaching constant weight. Subsequently, the samples were weighed and the values converted to g plant⁻¹. To obtain the N content in the leaves, the leaves of the plants obtained for previous analysis were removed and then placed in paper bags, duly identified and taken to the laboratory for analysis according to the methodology proposed by Sarruge and Haag (1974).

At harvest, 10 plants were collected at a predetermined location in the useful area of each plot and the following evaluations were performed: number of pods plant⁻¹, number of grains plant⁻¹, number of grains pod⁻¹. It was also evaluated the mass of 100 grains, determined by the random collection and weighing of two samples of 100 grains

per plot, and, finally, the grain yield, with the plants of the useful area of each plot being dried to full sun. After drying, they were submitted to mechanical trail and, later, the grains were weighed and the data transformed into kg ha⁻¹ (13% wet basis).

For the average cooking time of bean grains, determined by Mattson Cooker (CT), the analysis was performed following a method adapted from those proposed by Proctor and Watts (1997).

Data analysis

Data were subjected to analysis of variance by the F test. When the interaction between the evaluated factors was verified, the glyphosate factor was broken down for each nitrogen dose. Otherwise, if significant, regression analysis was performed for both factors using SISVAR version 5.3 software, calculated for linear and quadratic equations and considered significant at 5 (*) and 1 (**) % probability by the F test (Ferreira, 2011). When both were significant, the one with the highest determination coefficient (R²) was chosen. Then, to present the significant results in graph form, regression models were adjusted with the aid of SigmaPlot software.

Results and discussion

For the final plant population, it was possible to verify that there were no statistical differences (Table 2).

The common bean shoot dry matter mass data resulted interaction of variation factors only at 14 DAT (Table 2), with differentiated behavior of low glyphosate doses within each nitrogen dose (Figure 1).

In Figure 1 it is possible to observe the common bean dry matter mass values for glyphosate subdoses within two nitrogen doses (0 and 60 kg ha⁻¹). It is observed a characteristic behavior of the hormetic effect in the vegetative growth of the plants when in the absence of the nitrogen fertilizer, in the low doses of 7.2 and 14.4 g and ha⁻¹, in which the dry matter mass was 5, 9 g plant⁻¹ without glyphosate application up to 9.3 g plant⁻¹ with 14.4 g AE ha⁻¹. Already, when 60 kg ha⁻¹ of nitrogen is supplied to the common bean, the behavior is completely different, in which the glyphosate, since the smallest dose, affected the vegetative development of the crop, that is, expressed the herbicidal effect.

Rabello et al. (2015) concluded that glyphosate reduced the dry mass of cv. Pérola on drift occurrence at 20 DAA. In addition, working with young neem plants (*Azadirachta indica*), Yamashita et al. (2017) verified that for the dry mass of the aerial part of the plants, there was no difference between herbicide doses in the evaluation at 7 and 30 days, however, in the evaluation performed at 14 days, there was a progressive reduction in this variable, since, in the highest dose, a significant difference was observed in relation to the control. Nevertheless, as the results showed, the plants

recovered and at 30 days, there was no longer any difference between treatments. On the other hand, Nascentes et al. (2017) concluded that low doses of up to 10 g AE ha⁻¹ were able to increase sugarcane and eucalyptus dry mass at 40 and 60 DAA glyphosate.

Still regarding Figure 1, is evident that, upon confirmation of these results, a low glyphosate dose of approximately 15 g AE ha⁻¹ is able to replace around 60 kg ha⁻¹ of nitrogen for common bean crop vegetative development purposes. This proves to be quite significant when comparing the costs of each operation.

Table 2. Summary of analysis of variance of final plant population (PF), shoot dry matter mass (MS-1) at seven days after treatment (DAT) and at 14 DAT (MS-2), nitrogen (N) in leaves, plant height (AP) and stem diameter (DC) as a function of glyphosate subdoses and nitrogen doses in the irrigated winter common bean. Selvíria-MS, Brazil.

Variation factors	GL	Parameters					
		PF	MS-1	MS-2	N	AP	DC
Block	3	2,270 ^{n.s}	0,469 ^{n.s}	2,552 ^{n.s}	2,167 ^{n.s}	0,506 ^{n.s}	1,187 ^{n.s}
Glyphosate (G)	3	0,179 ^{n.s}	0,166 ^{n.s}	2,693 [*]	4,473 [*]	0,476 ^{n.s}	0,528 ^{n.s}
Error a	9						
Nitrogen (N)	4	1,502 ^{n.s}	1,267 ^{n.s}	0,794 ^{n.s}	0,506 ^{n.s}	0,119 ^{n.s}	1,139 ^{n.s}
G x N	12	1,040 ^{n.s}	1,583 ^{n.s}	2,125 [*]	2,940 ^{**}	0,711 ^{n.s}	1,743 ^{n.s}
Error b	48						
CV _a (%)		16,71	45,08	22,64	9,24	13,34	12,88
CV _b (%)		15,06	28,25	22,48	13,95	18,95	11,16
MG		9,94	6,12	8,07	6,76	94,46	5,13

** e * and n.s - significant at the 1% and 5% probability level by the F test and not significant, respectively; CV - coefficient of variation.

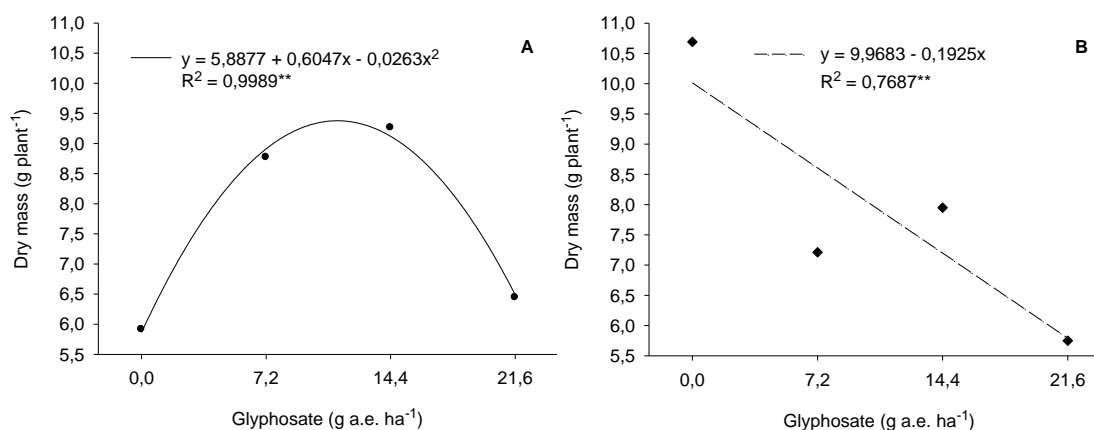


Figure 1. Interaction of low glyphosate doses within nitrogen [0 (A) and 60 (B) kg ha⁻¹] for dry matter mass of common bean at 14 DAT. Selvíria-MS, Brazil.

The summary analysis of variance in Table 2 shows that there was significant interaction at the 1% probability level of error for leaf N content. The N values found in all treatments were found to be adequate to those recommended for the culture (30 to 50 g kg⁻¹) to obtain high yields according to Ambrosano et al. (1997). This shows that herbicide subdoses did not cause significant damage to crop development.

The behavior observed in Figure 2 in relation to leaf N content in common bean is characterized as "inverted U" (Calabrese, 2007; Hashmi et al., 2014) and is one of the most common effects to be observed by the hormesis phenomenon.

Between 30 and 45 kg ha⁻¹ of N, the subdoses of 7.2 and 14.4 g AE ha⁻¹ were positive for

bean leaf N content (Figure 2). In the subdose of 21.6 g AE ha⁻¹, the process of falling in the contents of this nutrient seems to have been started, according to the quadratic equation generated from the obtained data. These results are similar to those obtained by Silva et al. (2016a) in this same common bean cultivar, where the low glyphosate dose of 15 g AE ha⁻¹ was higher than the control without application on leaf N content, ranging from 25.00 to 22.75 g N per kg of dry matter, respectively. Unlike Rabello, Monnerat and Vasconcelos Júnior (2014), who did not find significant differences in leaf N content of common bean BR1 Xodó with application of low glyphosate doses.

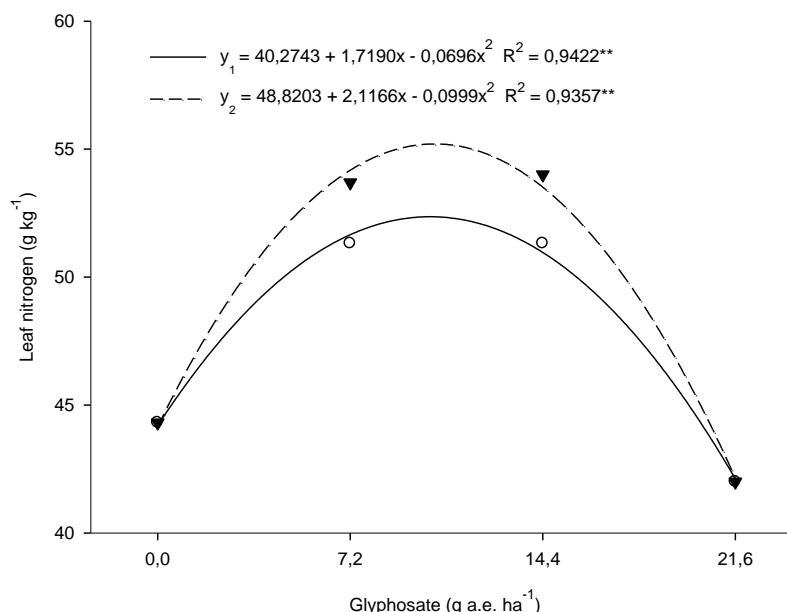


Figure 2. Interaction of low glyphosate doses within nitrogen [30 (○) and 45 (▼) kg ha⁻¹] for common bean leaf nitrogen content. Selvíria-MS, Brazil.

The vegetative components studied were plant height and stem diameter, as shown in Table 2. It was observed that both treatments were not influenced by the variation factors.

Regarding plant height, the results are consistent with those observed by Silva et al. (2012) who found no differences in this variable for the two years of research using subdoses of glyphosate ranging from 0 to 40 g active ingredient (AI) ha⁻¹. As with Maciel et al. (2009), who evaluated the use of low glyphosate doses at concentrations of 0.0; 11.2; 22.5; 45.0; 90.0; 180.0 and 360.0 g AE ha⁻¹ in white curauá plants (*Ananas erectifolius*) and concluded that none of the studied doses promoted stimulation or increase in plant vegetative development. However, Silva et al. (2009) found evidence that

early sugarcane development may be positively responsive to the application of subdoses of glyphosate, especially at doses around 1.8 g AE ha⁻¹. Similarly, Nascentes et al. (2015) found that the application of glyphosate subdoses increased dry matter yield, plant height and growth rate of *Brachiaria brizantha* cv. Marandu, and the best dose varied for each variable analyzed.

In the general context of the treatments, only glyphosate resulted in significant differences in the number of grains pod⁻¹ at the 5% probability level (Table 3). However, for the other parameters, such as NGP and NVP, there was no response. In addition, no interaction between the factors was verified.

Table 3. Summary of variance analysis of number pod plant⁻¹ (NVP), grains plant⁻¹ (NGP), grains pod⁻¹ (NGV), 100 grain mass (M100), yield (PROD) and average cooking time (CT) as a function of low glyphosate doses and nitrogen doses in irrigated winter common bean. Selvíria-MS, Brazil.

Variation factors	GL	Parameters					
		NVP	NGP	NGV	M100	PROD	TC
Block	3	2,305 ^{n.s}	2,138 ^{n.s}	0,289 ^{n.s}	0,772 ^{n.s}	7,441 ^{**}	0,002 ^{n.s}
Glyphosate (G)	3	0,519 ^{n.s}	0,597 ^{n.s}	2,359 ^{n.s}	0,168 ^{n.s}	5,131 [*]	2,227 ^{n.s}
Error a	9						
Nitrogen (N)	4	0,707 ^{n.s}	1,020 ^{n.s}	1,178 ^{n.s}	0,662 ^{n.s}	3,091 [*]	2,018 ^{n.s}
G x N	12	0,676 ^{n.s}	1,580 ^{n.s}	0,966 ^{n.s}	1,292 ^{n.s}	2,860 ^{**}	9,947 ^{**}
Error b	48						
CV _a (%)		18,66	25,56	22,30	7,74	9,37	13,28
CV _b (%)		25,49	25,55	21,04	6,22	10,29	7,03
MG		10,01	48,69	4,92	25,13	2482,91	19,53

** e * and n.s - significant at the 1% and 5% probability level by the F test and not significant, respectively; CV - coefficient of variation.

According to the summary analysis of variance contained in Table 3, it can be observed that the 100 grain mass was not influenced by any of the treatments. With an average value of 25.13 g. Corroborating the results of Silva et al. (2013b) in the culture of the sorghum cultivar BRS 310 in which

the use of low glyphosate doses of 0 g ha⁻¹, 15 g ha⁻¹, 30 g ha⁻¹, 45 g ha⁻¹, 60 g ha⁻¹ and 75 g ha⁻¹ of the acid equivalent did not provide significant variation in the 1000 grain mass.

Silva et al. (2012) observed the highest glyphosate subdoses provided a significant

decrease in the 100 grain mass, with the results adjusted to a decreasing linear equation.

The same was verified by Melhorança Filho et al. (2010), in the conventional soybean crop, indicating that as the doses increased (0 g ha⁻¹, 5 g ha⁻¹, 10 g ha⁻¹, 15 g ha⁻¹, 20 g ha⁻¹, 25 g ha⁻¹ and 30 g ha⁻¹ of AI), there was a linear decrease in grain mass. Unlike results obtained by Magalhães et al. (2001), simulating glyphosate and paraquat herbicide drift, as well as their effects on plant development and sorghum grain yield, demonstrated that the 1000 grain mass was not affected by the low herbicide doses.

There was a significant interaction of factors N and G for common bean yield (Table 3). For a better visualization of the results, the bean yield values for the significant results of the interaction of glyphosate within nitrogen doses are shown below (Figure 3). As can be seen this occurred at the doses of 45 and 60 kg ha⁻¹ of N.

In the first case, with 45 kg ha⁻¹ N, the regression was in inverted U format, as described by Calabrese (2007) and Hashmi et al. (2014). In this context, the lowest doses (7.2 and 14.4 g ha⁻¹) of glyphosate active ingredient were favorable to

common bean yield, reaching values close to 2,850 kg ha⁻¹ in the 14.4 g ha⁻¹ and subsequently there was a fall with the subdose of 21.6 g AE ha⁻¹ to values below the control. In contrast, with 60 kg ha⁻¹ of N the exact opposite occurred, with the regression curve behaving in a "J" format as described by Hashmi et al. (2014). Thus, the lower subdoses (7.2 and 14.4 g ha⁻¹) of the active ingredient glyphosate were detrimental to common bean yield, and thereafter there was a resumption with 21.6 g ha⁻¹ for values close to the control treatment, which was 2.350 kg ha⁻¹.

In Figure 3 it is also possible to notice the great influence of nitrogen on common bean yield, since, in the absence of glyphosate, the increase of 15 kg ha⁻¹ from 45 to 60 kg ha⁻¹ N promoted increment of more than 400 kg of grains per hectare. However, it is noted that the use of 14.4 g ha⁻¹ of glyphosate accompanied by 45 kg ha⁻¹ of N is able to bring similar results to the productivity obtained with N alone in the 60 kg ha⁻¹. This confirms the hypothesis raised in this paper that nitrogen or nutrient availability may affect the glyphosate hormesis results in common bean crop.

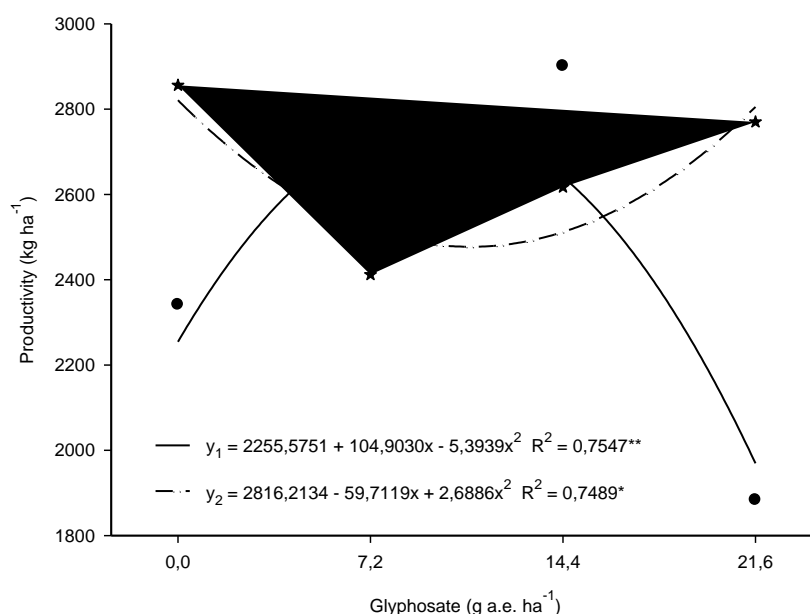


Figure 3. Interaction of glyphosate subdoses within nitrogen [45 (●) and 60 (*) kg ha⁻¹] for common bean yield. Selvíria-MS, Brazil.

Still regarding productivity, the results obtained at the dose of 45 kg ha⁻¹ of N were similar to those of Silva et al. (2012), where Juriti and Pérola common bean cultivars responded positively to glyphosate in the 10 g AI ha⁻¹, but the same did not occur with the early Carioca Precoce cultivar. In cultivar Juriti the gains were 7.5 to 10% in relation to control without application.

Cedergreen et al. (2009) obtained an increase in barley grain yield (12-15%), with the application of glyphosate subdoses between 2.5 g ha⁻¹ and 20 g ha⁻¹ of the active ingredient. On the

other hand, results obtained by Magalhães et al. (2001), simulating glyphosate and paraquat herbicide drift, as well as their effects on plant development and sorghum grain yield, showed gains of approximately 400 kg ha⁻¹ in sorghum productivity, when sub-doses between 2% and 4% of the recommended dose of the commercial product were used.

Given the results obtained in bean yield and nutrition by the hormetic effect of glyphosate, many doubts arise as to the other characteristics that may be influenced, among which, the technological quality is highlighted by the importance in the

acceptance of the product by the consumer. Thus, tests were performed with Mattson Cooker to analyze the average cooking time (CT), which

resulted in the interaction between glyphosate and nitrogen that is represented in Figure 4.

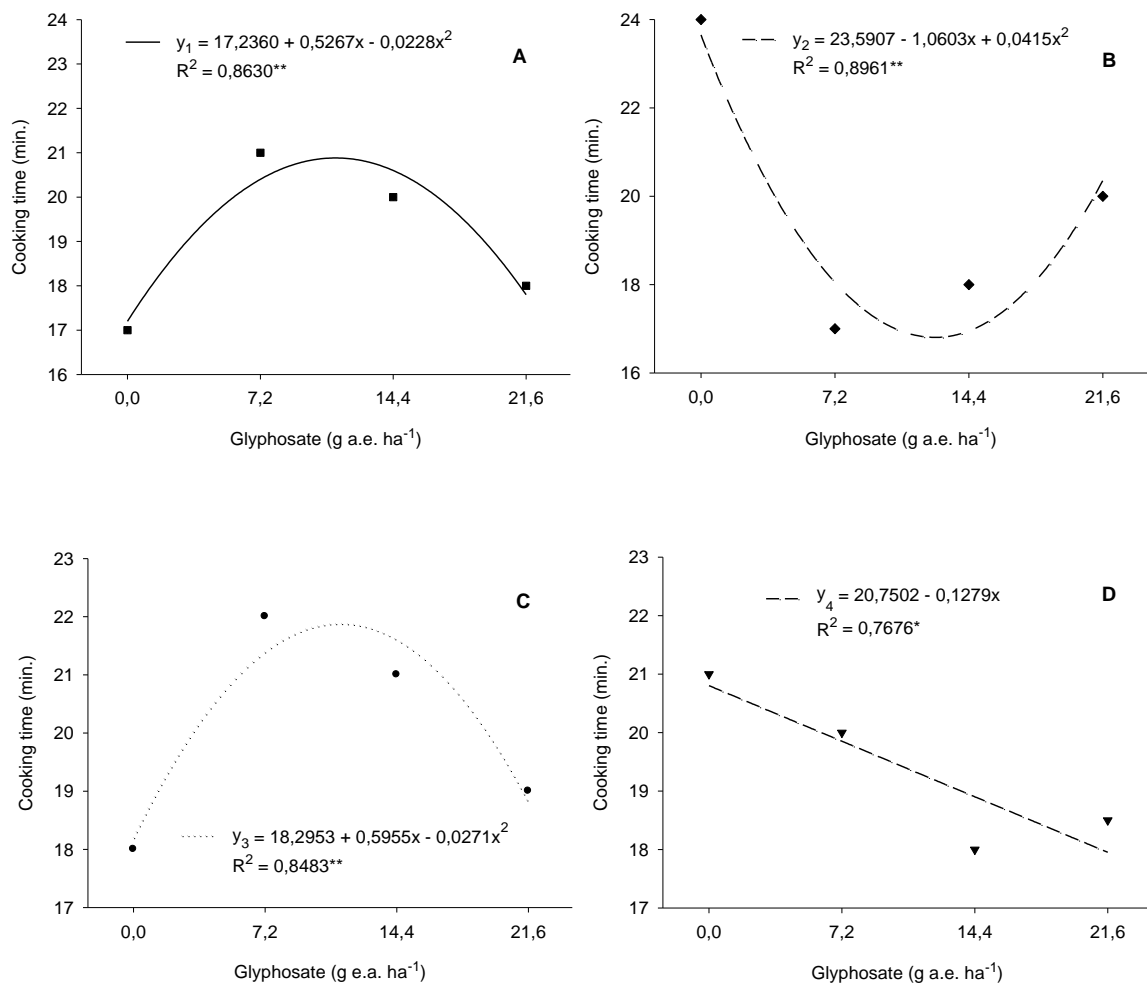


Figure 4. Interaction of glyphosate subdoses within nitrogen [0 (A); 30 (B); 45 (C) and 60 (D) kg ha⁻¹] for the cooking time of common bean grains. Selvíria-MS, Brazil.

Except for the 15 kg ha⁻¹ N dose, all were affected by glyphosate subdoses with respect to the average cooking time. Proving once again that there may be interference of nitrogen fertilization on the results of hormesis even in the quality of the grains.

In the absence of cover N (Figure 4 A) and application of 45 kg ha⁻¹ (Figure 4 C) the CT was higher within 4 minutes for the lowest herbicide subdoses (7.2 and 14.4 g ha⁻¹) with a normalization

Conclusion

The range of 10 to 12 g A.E. ha⁻¹ of glyphosate generated better results in the vegetative components, in the productivity and in the average cooking time of the common bean.

There was an increase in the productivity of beans by 500 kg ha⁻¹ or 21% in relation to the control in the estimated subdose of 10 g A.E. ha⁻¹ and a reduction in nitrogen by 15 kg ha⁻¹.

Acknowledgment

tendency in the subdose of 21.6 g ha⁻¹, following the quadratic behavior characterized by the inverted U format, as described by Calabrese (2007) and Hashmi et al. (2014). However, at doses of 30 and 60 kg ha⁻¹ of N (Figures 4 B and D), it was observed that the low glyphosate doses reduced CT up to 6 minutes compared to the control without application, with quadratic behavior for the dose is 30 linear and decreasing for the dose of 60 kg of N per hectare.

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