

Hormetics effects of glyphosate on coffee plants – guidelines for coffee

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ABSTRACT

Glyphosate stands out among non-selective herbicides used for management of weeds in coffee crops due to its favorable physical-chemical, economical, and environmental characteristics. The hormetic effect is the tonic effect of low doses of toxic substances, such as herbicides, on plants. The present study was conducted at the IFSULDEMINAS, Muzambinho campus, Minas Gerais, Brazil, to identify the hormetic effect of glyphosate on coffee seedlings (*Coffea arabica* cv. Catuaí Vermelho 144), using different low doses of the herbicide. The experiment was conducted in a randomized block design. The treatments consisted of 8 glyphosate herbicide low doses (0, 22.5, 45, 90, 180, 360, 720, and 1,440 g ha⁻¹) distributed in three blocks, containing ten plants per plot, using a two-plant border between treatments, totaling 240 plants. The plant development over time was simulated using non-linear models. Growth models were adjusted using the Lorentz model to analyze the regression with four parameters. Stem diameter, plant height, and total leaf area were evaluated from 7 to 63 days after the herbicide application. Hormetic effect on the coffee seedlings evaluated was found when using the low doses of 22.5 g ha⁻¹ and 45 g ha⁻¹, and the best effect was found with the low dose of 22.5 g ha⁻¹. Low doses of 300 g ha⁻¹ glyphosate stimulated the sprouting of coffee plants, and this dose is interesting for clonal seedlings.

Key words: Chemical control; *Coffea spp.*; herbicide; phytotoxicity; low doses.

1 INTRODUCTION

Herbicides are part of a commercial class of products consisted of mixtures of substances that form agrochemical formulations; they consist of active, inert, and sometimes, adjuvant ingredients. Herbicides contain toxic substances that can kill susceptible plant populations when applied at specific doses.

Glyphosate is an emerging post herbicide, recommended for total vegetation control, with systemic action, simplastic translocation and belonging to the replaced glycine chemical group. The mechanism of action is the inhibition of the enzyme EPSPs in the synthesis route of aromatic amino acids tyrosine, tryptophan and phenylalanine, precursors of lignin, flavonoids and benzoic acids (Kruse; Tressi; Vidal, 2000).

Glyphosate is not a selective herbicide, thus, there is a risk of damages to crops due to non-intentional exposure of the crop to the herbicide spray drift (Schrubbers et al., 2014). Spray drift is an undesirable effect of agrochemical applications; it is caused by the carrying of small drops by the wind to non-target locations, causing environmental contamination, and toxicity to plants in the case of herbicides (Miranda et al., 2016).

The amount of herbicide applied that can reach non-target plants by spray drift is estimated in up to 10% (Eker et al., 2006; Cedergreen, 2008; Al-Khatib et al., 2003; Asman et al., 2003); however, this percentage may be even higher (Felix; Boydston; Burke, 2011).

The vegetative and metabolic development of coffee plants can be affected by very low doses of herbicides through spray mists that reach coffee crops (Schrubbers et al., 2014).

The use of toxic substances in herbicides at very lower doses than those commercially recommended for killing plants can stimulate the plant development or cause other benefits; this effect is known as hormesis or hormetic effect (Calabrese; Baldwin, 2002).

Brito et al. (2017) reported that hormetic herbicide low doses are often between 5 and 10% of the commercially recommended dose and cause vegetative stimuli of approximately 20% to 30% (Schrubbers et al., 2014; Brito et al., 2017; Cedergreen; Ritz; Treibig, 2005). Effects of up to 160% over the control response are common, but above 200% are seldom (Agathokleous; Calabrese, 2020; Calabrese et al., 2019).

Studies have shown that the application of low low doses of toxic products to plants, such as glyphosate, can be stimulators for several crops and can improve their vegetative development with significant gains by increasing production and quality of the products, as found for maize (Cedergreen, 2008), barley (Meseldzija et al., 2020), pine (Mollae et al., 2020), chickpea (Abbas et al., 2015), eucalyptus (Nascentes et al., 2018), faba bean (El Shahawy; Sharara, 2011), tomato (khan et al., 2020), sugarcane (Silva et al., 2009), soybean (Velini et al., 2008), common bean (Silva et al., 2012), rice

(Ferrari et al., 2021a; Ferrari et al., 2021b) and *Cattleia nobilior* (Bertoncelli et al., 2018).

Moreover, greater vegetative development of coffee plants was found using glyphosate low doses of 57g ha⁻¹ and 115.2 g ha⁻¹ (França et al., 2010).

Schrubbers et al. (2014) found hormesis in coffee plants for low glyphosate low doses (11, 28, and 69 g ha⁻¹), in a field experiment.

It is also important to mention that hermetic effects can be compromised due to several factors such as extremely low doses, below symptomatic levels for plants; or when applied in dry seasons, hindering the penetration and mobility of the herbicide in plants; or plant age directly interfering with the a.i. ratio of the herbicide *versus* dry mass of the plant; or, as the herbicide is applied in the plant, spraying being the best way to observe the symptoms of hormesis effect due to the ease of herbicide dosage control.

Several factors prevent the consolidation of the use of the hormesis technique in agriculture such as the time of herbicide application, the molecule to be used, the correct low dose, the stage of the plant for the application and the mode of application of the herbicide for the observance of the expected hermetic effects.

Therefore, the objectives of this work were to apply best statistic models to estimate the hormetic effect of applications of glyphosate low doses to coffee plants; identify the best herbicide low dose for the hormetic effect, considering the

evaluated characteristics; and describe the toxicity symptoms based on observations of morphological characteristics of recently implemented coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) within the different glyphosate low doses. The hypothesis was that the lowest doses cause a significant hormetic effect to young coffee plants and surviving coffee plants can recover from toxicity caused by glyphosate within 2 months.

2 MATERIAL AND METHODS

The experiment was carried out at the fruit production sector of the Federal Institute of Education, Science, and Technology of Southern Minas Gerais (IFSULDEMINAS), Muzambinho campus (21°20'54.19"S, 46°31'36.22"W, and altitude of 1014 meters), Minas Gerais, Brazil. The soil of the area was classified as a Typic Hapludox (Typical dystroferic red yellow latosol) (Antunes et al., 2018). The region presents a Cwb, subtropical highland climate, with rainy summer and mild dry winter, according to the climate classification of Köppen (1948). The used area were 2.80 m width and 80 m length with 15% slope (Figure 1).

The experiment was carried out in a randomized block design, with 8 glyphosate herbicide low doses (0, 22.5, 45, 90, 180, 360, 720, and 1,440 g ha⁻¹) distributed in three blocks, containing ten plants per plot, using a two-plant border between treatments, totaling 240 plants.

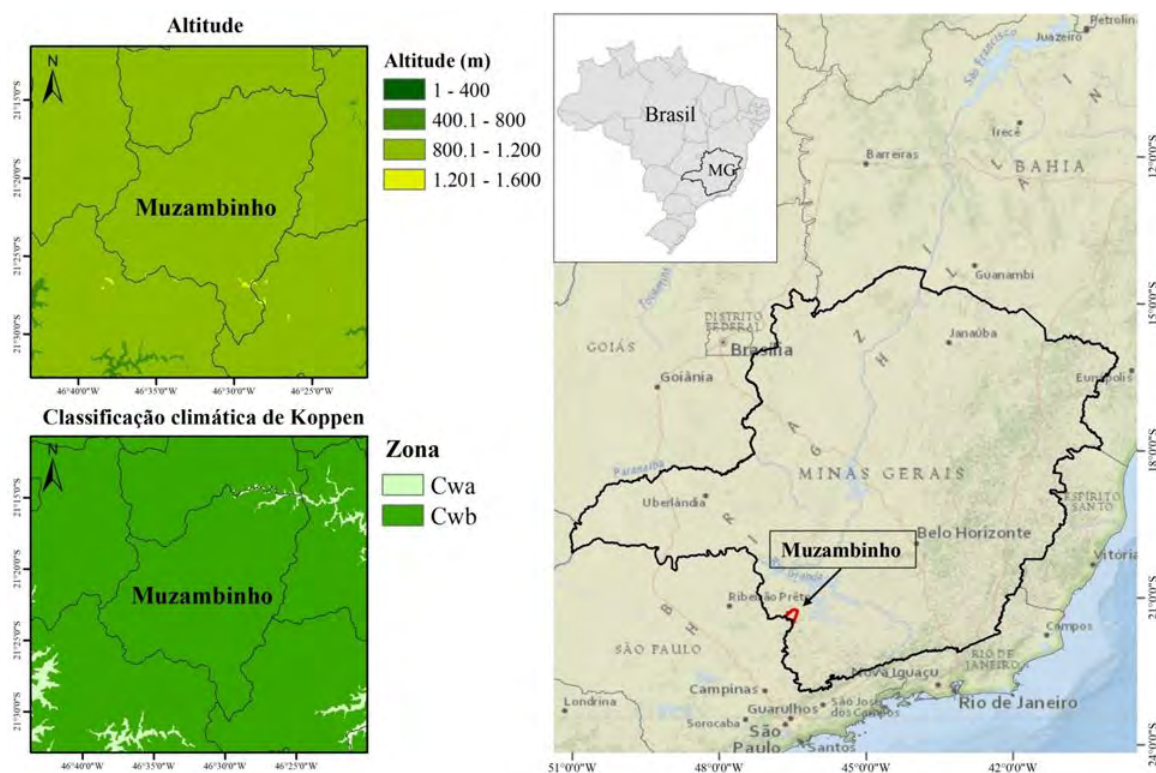


Figure 1: Location of the municipality of Muzambinho in the Alta Mogiana region and in the state of Minas Gerais, Brazil.

Seedlings of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) were planted on May 28, 2018, after subsoiling, furrowing, liming, and application of soil fertilizer, according to laboratorial soil analysis (Table 1). The seedlings had four pairs of expanded leaves with more than 2 cm length. The plant spacing used was 2.8 m × 0.6 m.

Weeds were controlled through manual weeding until the herbicide application. The glyphosate herbicide (Roundup Original®) was applied on October 2, 2018 in the morning period to promote a better absorption.

The established low doses were applied using a CO₂-pressurized backpack sprayer (Herbicat®) with a spray boom containing two air-induction nozzles (110-02) spaced 0.5 m apart, using a solution volume equivalent to 250 L ha⁻¹, spray pressure of 3 bar (43.5 psi), and application speed of 3.60 km h⁻¹. The climate data at the time of application, acquired using a thermohygrometer (Herbicat®), showed an air temperature of 29 °C and a relative air humidity of 79%.

The vegetative growth of coffee plants was evaluated at 7, 21, 35, 49, and 63 days after application (DAA) of the herbicide. The parameter evaluated were: plant height, from the stem base to the meristematic apex; stem base diameter, measured with a caliper (Miranda et al., 2006); number of sprouts; and total leaf area (TLA) (Silva; Leite; Ferreira, 2008), measured using the equations: Leaf area (LA) = leaf length × leaf width × 0.663 (correction factor), and TLA = ∑LA. One plant per plot was collected for this destructive analysis.

The data of plant height, stem diameter, total leaf area, and number of sprouts were evaluated through analysis of variance, and the means were compared by the Scott-Knott test at 5% significance level, using the Sisvar 5.6 program (Ferreira, 2011).

The plant development over time was simulated using non-linear models. Growth models were adjusted using the Lorentz model to analyze the regression with four parameters (Equation 1). The normal distribution of deviations was found using the adhesion test of Kolmogorov-Smirnov at $p < 0.05$ (Aparecido; Rolim, 2020).

$$y = y_{\min} + \frac{2A}{\pi} \frac{w}{4(x - x_0)^2 + w^2} \quad (1)$$

where $y_{\min}$ is the minimum development in the curve, which tends to coincide with the highest efficiency of the product;

X_0 is the glyphosate low dose at the highest plant vegetative development; A is the total area under the development curve; and W is the standard deviation of the midpoint or total width of the peak at half height, as indicated by Hinnah et al. (2018).

The observed field data were compared to the results of all models using several statistical indexes of precision and significance level. The accuracy indicates the proximity of an estimate to the observed value; it was evaluated using the mean absolute percentage of error (MAPE) (Equation 2). The precision is the ability of a model to repeat the estimate; it was evaluated using the adjusted coefficient of determination (R^2) (Cornell; Berger, 1987) (Equation 3).

$$MAPE = \frac{\sum_{i=1}^N \left(\left| \frac{Yest_i - Yobs_i}{Yobs_i} \right| * 100 \right)}{N} \quad (2)$$

$$R^2_{ajustado} = \left[1 - \frac{(1 - R^2) \times (N - 1)}{N - k - 1} \right] \quad (3)$$

3 RESULTS

3.1 Vegetative growth after herbicide application

The means of height of coffee plants grown under the different glyphosate low doses applied (Table 2) showed significant difference at 7 days after application (DAA) of the herbicide. The herbicide low dose of 22.5 g ha⁻¹ presented significant difference from 21 to 35 DAA, but with no difference from the doses lower than 45 g ha⁻¹ from 49 to 63 DAA.

The means of stem diameter of coffee plants subjected to different glyphosate low doses are shown in Table 3. Hormetic effect was found from 35 DAA for the low dose of 22.5 g ha⁻¹, presenting significant differences up to 63 DAA, and showing gains of 20% to 30%, as also reported by Brito et al. (2017), presenting values little below 20% in the last evaluation.

The means of total leaf area of coffee plants (Table 4) showed that doses lower than 360 g ha⁻¹ presented significant differences from the control only at 21 DAA; at 35 DAA, doses lower than 45 g ha⁻¹ show higher total leaf area than the other low doses and similar to the control; at 63 DAA, only the low dose of 22.5 g ha⁻¹ resulted in total leaf area similar to the control and higher than that found using the other low doses.

Table 1: Chemical analysis of the soil where the coffee seedlings (*Coffea arabica* cv. Catuaí Vermelho 144) were planted. Muzambinho, MG, 2018.

pH	P	K	Zn	B	Mn	Fe	Ca	Mg	Al	H + Al	SB	t	T	M	V
H ₂ O	mg dm ⁻³							cmol _c dm ⁻³				%			
5.1	10.2	77	2.6	0.15	11.2	16.7	3.31	1.22	0.07	3.75	4.7	4.8	8.5	1.5	55.8

Laboratory of soil analysis of Federal Institute of Education, Science, and Technology of Southern Minas Gerais, Muzambinho campus.

Table 2: Height (cm) of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

Dose (g ha ⁻¹)	Days after application (DAA)										Mean
	7 DAA		21 DAA		35 DAA		49 DAA		63 DAA		
	cm	%	cm	%	cm	%	Cm	%	cm	%	
0	27.53A	100	31.67B	100	31.10B	100	31.00A	100	34.07A	100	31.07B
22.5	28.93A	105.08	34.00A	107.37	34.83A	112	33.47A	107.96	34.63A	101.66	33.17A
45	28.40A	103.15	31.20B	98.53	31.20B	100.32	31.93A	103.01	34.57A	101.47	31.46B
90	29.07A	101.69	30.40B	96	28.87C	92.82	29.37B	94.73	32.33B	94.91	30.01C
180	27.13A	98.55	28.00C	88.55	28.83C	92.71	28.93B	93.33	32.13B	94.32	29.01C
360	28.17A	102.30	28.43C	89.79	28.17C	90.57	29.87B	96.34	31.47B	92.37	29.22C
720	28.47A	103.39	26.33C	83.16	28.07C	90.25	28.83B	93.01	30.43B	89.33	28.43C
1440	26.70A	96.97	27.30C	86.21	27.47C	88.32	25.53C	82.37	31.40B	92.17	27.68C
Mean	28.05 c		29.67 b		29.82 b		29.87 b		32.63 a		30.01
CV-1 (%)	7.18										
CV-2 (%)	5.08										
F _{doses_overall}	0.0001										
F _{dose}	0.6089		0.0000		0.0000		0.0000		0.0144		

Results of the F test and test of means. Means followed by same lowercase letter in the rows, or uppercase letter in the columns, are not different by the Scott-Knott test at 5% significance level.

Table 3: Stem diameter (mm) of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

Dose (g ha ⁻¹)	Days after application (DAA)										Mean
	7 DAA		21 DAA		35 DAA		49 DAA		63 DAA		
	mm	%	mm	%	mm	%	Mm	%	Mm	%	
0	0.40A	100	0.50A	100	0.50B	100	0.50B	100	0.68B	100	0.52B
22.5	0.40A	100	0.55A	110	0.62A	123.33	0.63A	126.67	0.80A	117.07	0.60A
45	0.37A	91.67	0.50A	100	0.50B	100	0.50B	100	0.63B	92.68	0.50B
90	0.42A	104.17	0.47A	93.33	0.45B	90	0.47B	93.33	0.58B	85.37	0.48B
180	0.40A	100	0.48A	96.67	0.47B	93.33	0.47B	93.33	0.63B	92.68	0.49B
360	0.42A	104.67	0.50A	100	0.43B	86.67	0.43B	86.67	0.65B	95.12	0.49B
720	0.43A	108.33	0.43A	86.67	0.40B	80	0.42B	83.33	0.57B	82.93	0.45B
1440	0.38A	95.83	0.42A	83.33	0.43B	86.67	0.38B	76.67	0.52B	75.61	0.43B
Mean	0.40 c		0.48 b		0.48 b		0.48 b		0.63 a		0.49
CV-1 (%)	18.90										
CV-2 (%)	14.18										
F _{doses_overall}	0.0074										
F _{dose}	0.9770		0.4780		0.0318		0.0076		0.0013		

Results of the F test and test of means. Means followed by same lowercase letter in the rows, or uppercase letter in the columns, are not different by the Scott-Knott test at 5% significance level.

The results of the test of means showed that the total leaf area of plants under the herbicide low dose of 22.5 g ha⁻¹ was always higher than 20% when compared to that of plants of the control, except for 35 DAA (Table 4 and Figure 2), confirming the results found by Brito et al. (2017), which indicate hormesis.

The means of number of lateral sprouts of coffee plants (Table 5) showed no significant difference for the herbicide low doses and evaluation times tested, except for 49 and 63 DAA, when the low dose of 360 g ha⁻¹ resulted in plants with higher number of sprouts than the other low doses, and for 35 DAA, when the lowest herbicide low doses resulted in similar

results to the control and lower results than the low doses higher than 90 g ha⁻¹.

3.2 Lorentz model for the hormetic effect

In Figure 2, all the columns for the development of the analyzed characteristics are presented. The plant development, according to the characteristics analyzed, showed that the

plant height, stem diameter, and total leaf area presented better results when using the lowest glyphosate low doses, with peaks at similar points. However, the number of sprouts showed a peak at the low dose of 360 g ha⁻¹.

Plant height and stem diameter were higher at 63 DAA in all tested low doses, showing that the effect of glyphosate on the plant decreased within 2 months after the herbicide application.

Table 4: Total leaf area (cm²) of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

Dose (g ha ⁻¹)	Days after application (DAA)										Mean
	7 DAA		21 DAA		35 DAA		49 DAA		63 DAA		
	cm ⁻²	%	cm ⁻²	%	cm ⁻²	%	cm ⁻²	%	cm ⁻²	%	
0	2.13A	100	2.14B	100	2.54A	100	2.40A	100	2.26A	100	2.30A
22.5	2.28A	129.56	2.39A	171.11	2.43A	77.87	2.57A	148.89	2.38A	133.73	2.41A
45	2.27A	125.61	2.29A	135.63	2.45A	81.93	2.32A	85.68	2.02B	73.26	2.27A
90	2.28A	129.17	2.36A	157.43	2.24B	50.59	2.09B	51.99	1.90C	45.08	2.17B
180	2.05A	87.82	2.25A	122.51	2.17B	43.23	2.02B	42.43	1.93C	48.96	2.09B
360	2.23A	122.63	2.31A	140.79	2.13B	39.36	2.05B	46.37	2.06B	67.35	2.16B
720	2.20A	109.63	2.15B	97.21	2.20B	45.41	2.02B	41.77	1.53 D	18.80	2.02C
1440	2.17A	104.94	1.97B	69.20	2.11B	38.79	1.92B	34.75	1.80C	37.14	1.99C
Mean	2.20 a		2.23 a		2.28 a		2.17 a		1.99 b		2.18
CV-1 (%)	7.57										
CV-2 (%)	6.55										
F _{doses_overall}	0.0001										
F _{doses}	0.5254		0.0163		0.0017		0.0000		0.0000		

Results of the F test and means transformed into log(x). Means followed by same lowercase letter in the rows, or uppercase letter in the columns, are not different by the Scott-Knott test at 5% significance level.

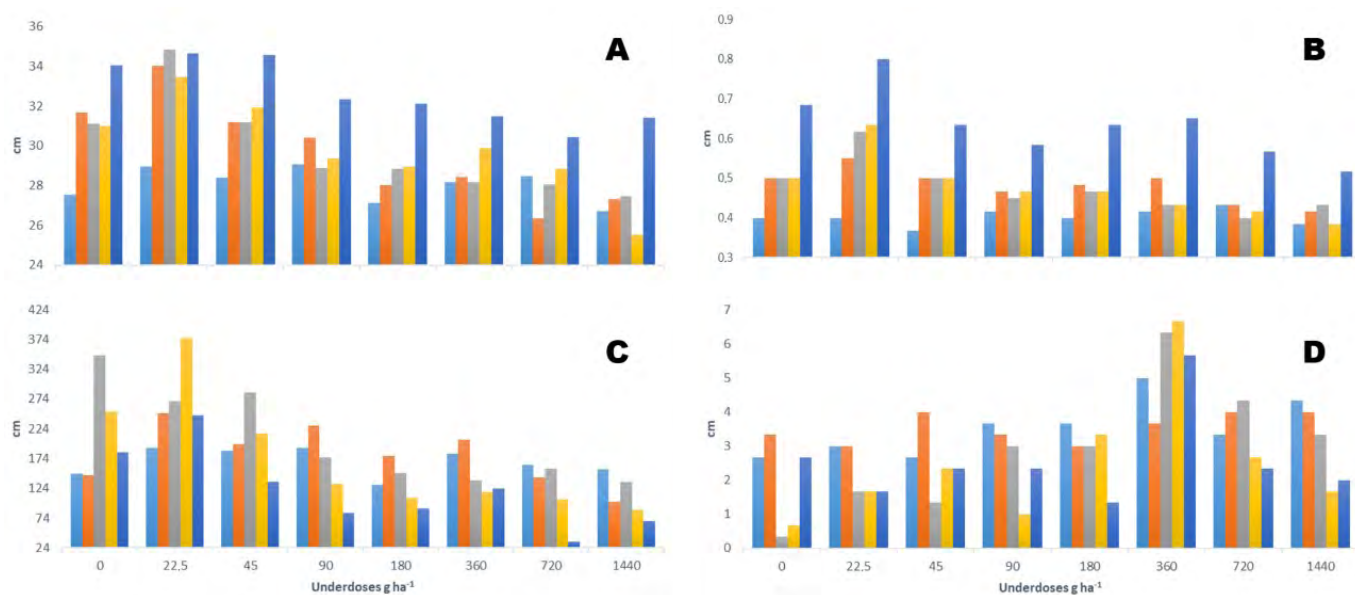


Figure 2: Plant height (A), stem diameter (B), total leaf area (C), and number of sprouts (D) of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144), evaluated at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

Table 5: Number of sprouts of coffee plants (n) (*Coffea arabica* cv. Catuaí Vermelho 144) at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

Dose (g ha ⁻¹)	Days after application (DAA)										Mean
	7 DAA		21 DAA		35 DAA		49 DAA		63 DAA		
	n	%	N	%	n	%	N	%	n	%	
0	1.88 A	100	1.96 A	100	1.00 B	100	1.28 B	100	1.87 A	100	1.60 B
22.5	1.88 A	NE	1.89 A	90	1.55 B	77.87	1.58 B	250	1.58 A	62.5	1.69 B
45	1.88 A	NE	2.23 A	120	1.47 B	81.93	1.80 B	350	1.80 A	87.5	1.84 B
90	2.15 A	NE	2.08 A	100	1.99 A	50.59	1.38 B	150	1.73 A	87.5	1.87 B
180	2.14 A	NE	1.99 A	90	1.96 A	43.23	2.07 B	500	1.47 A	50	1.93 B
360	2.39 A	NE	2.14 A	110	2.70 A	39.36	2.76 A	1000	2.56 A	212.5	2.51 A
720	2.03 A	NE	2.23 A	120	2.29 A	45.41	1.90 B	400	1.73 A	87.5	2.04 B
1440	2.29 A	NE	2.09 A	120	1.94 A	38.79	1.63 B	250	1.67 A	75	1.92 B
Mean	2.08 a		2.07 a		1.86 b		1.80 b		1.80 b		1.92
CV-1 (%)	27.84										
CV-2 (%)	25.72										
F _{doses_overall}	0.0139										
F _{doses}	0.8609		0.9877		0.0045		0.0187		0.2595		

Results of the F test and means transformed into log(x). Means followed by same lowercase letter in the rows, or uppercase letter in the columns, are not different by the Scott-Knott test at 5% significance level.

In Figure 3 shows the results of the Lorentz model for the variable height of coffee plants subjected to underdosing of glyphosate and Table 6 shows the values of the Lorentz model with the correlation coefficient (R^2) and MAPE for the height variable of plants.

The Lorentz model for plant height showed peaks at 21, 35, and 49 DAA (Figure 3); however, the coefficient of correlation (R^2) and mean absolute percentage of error (MAPE) presented a good fit to the curves (Table 6).

In Figure 4 shows the results of the Lorentz model for coffee stem diameter subjected to glyphosate underdosing and Table 6 shows the values of the Lorentz model with the correlation coefficient (R^2) and MAPE for coffee stem diameter.

The Lorentz model for stem diameter showed peaks at 21, 35, 49, and 63 DAA (Figure 4); however, R^2 and MAPE presented a good fit to the curves (Table 6), with the lowest value at 21 DAA ($R^2 = 0.62$).

In Figure 5 shows the results of the Lorentz model for the total leaf area of coffee plants subjected to underdosing of glyphosate and table 6 shows the values of the Lorentz model with the correlation coefficient (R^2) and MAPE for the area total leaf.

The Lorentz model for total leaf area showed peaks at 7, 21, 49, and 63 DAA (Figure 5); however, R^2 and MAPE presented a good fit to the curves (Table 6), with coefficients of correlation above 85% at 35 DAA.

In Figure 6 shows the results of the Lorentz model for number of coffee shoots subjects to underdosing of glyphosate and

Table 6 shows the values of the Lorentz model with the correlation coefficient (R^2) and MAPE for number of coffee shoots.

The Lorentz model for number of sprouts of coffee plants showed peaks at 7, 35, 49, and 63 DAA (Figure 6); however, R^2 presented a good fit to the more distant curves of herbicide application (Table 6), with coefficients of correlation above 85%, and the accuracy (MAPE) showed a large variation.

The values of X0 for plant height, stem diameter, and total leaf area were close to the herbicide low dose of 22.5 g ha⁻¹, denoting a good fit of the model to assess the hormetic effect on coffee plants of the cultivar Catuaí Vermelho IAC-144 (Table 6). X0 indicates the X value (dose) at the development peak for the low doses estimated in the Lorentz model.

3.3 Symptoms of glyphosate toxicity in coffee plants

The toxicity of glyphosate (180 and 360 g ha⁻¹) on coffee plants at 63 DAA (Figure 7) showed a light chlorosis in young leaves and a light leaf narrowing and chlorosis in leaf blades. However, the symptoms become practically imperceptible when using herbicide low doses of 180 and 360 g ha⁻¹, showing that the plants were practically fully recovered at 63 DAA.

The symptoms found in the present work were: discoloration of recently formed leaves, leaf deformation, leaf chlorosis, and chlorosis of the leaf blade for the lowest glyphosate doses; and formation of narrow leaves, darkening of old leaves, and stem overgrowth for the highest glyphosate low doses.

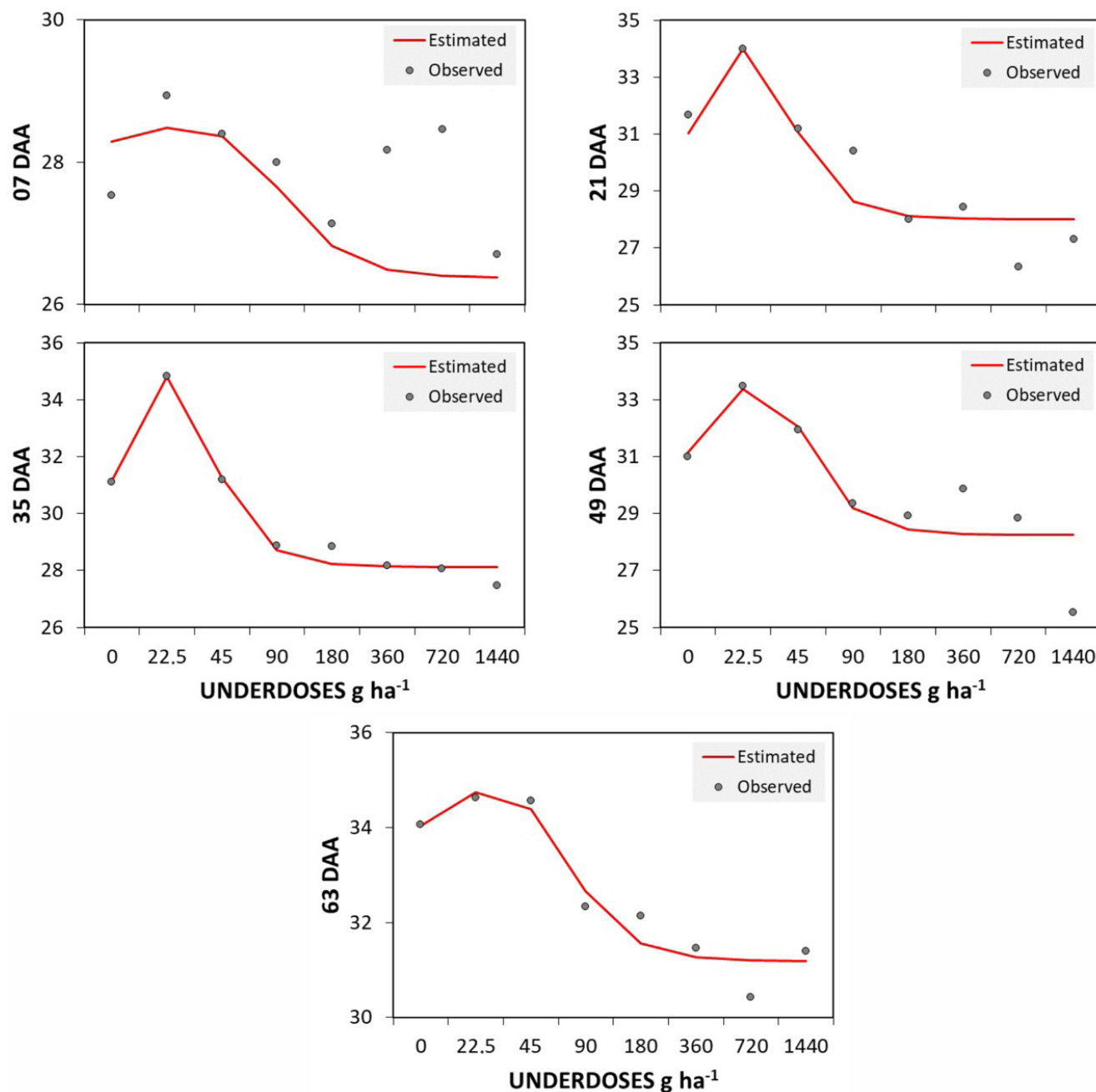


Figure 3: Lorentz model for data of plant height of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) evaluated at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

The toxicity symptoms of glyphosate, at low doses of 720 g ha⁻¹ and 1,440 g ha⁻¹, in coffee plants at 63 DAA (Figure 8) were: chlorosis, formation of narrow leaves, darkening of old leaves, and stem overgrowth.

The highest intensity of symptoms of toxicity of glyphosate at the low doses of 720 and 1440 g ha⁻¹ was found at 63 DAA; thus, the symptoms will probably last longer in the coffee plants that were subjected to the highest glyphosate low doses.

The symptom of acute toxicity of glyphosate in coffee plants is seen at the end of the plagiotropic or orthotropic branch, whose internode become short, in association with formation of leaves with narrow leaf blades, showing a narrow overgrowth aspect (Figure 9).

The end of the glyphosate effect occurs after the leaf enlargement and chlorosis and increase of the internodes in plagiotropic and orthotropic branches; the leaves gradually return to normal in next leaf formations (Figure 9). The end

of the glyphosate effect also depends on the low dose used; plants under smaller glyphosate low doses present earlier recovery than those under higher low doses.

4 RESULTS

4.1 Vegetative growth after herbicide application

According to Schrubbers et al. (2016), the metabolism of glyphosate in superior plants is slow and the herbicide is not fully metabolized in short periods of time, which was confirmed by the different results found at 7 DAA throughout the experiment.

Brito et al. (2017) reported that the use of low doses between 5% and 10% of the commercially recommended low dose of the herbicide can provide gains of 20% to 30% in crops sensitive to hormesis. In the present work, the highest gains for plant height were found at 35 DAA, presenting values above 12% (Table 2); thus, despite the significant gains, the values were not within those reported by these authors.

França et al. (2010) tested the toxicity of glyphosate at different low doses to coffee plants (cultivars Topázio, Catucaí, and Oeiras) and found a quadratic trend for plant height as the glyphosate low dose was decreased, with a maximum estimated plant height with a glyphosate low dose of 116.49 g ha⁻¹. However, they used only four glyphosate low doses, whose lowest low dose was 57.6 g ha⁻¹, which is higher than the lowest one used in the present work (22.5 g ha⁻¹). Thus, only the effect of higher low doses of the herbicide is known, characterizing a gradual toxicity to coffee plants and making the hormetic effect on coffee plant growth imperceptible when using doses lower than 57.6 g ha⁻¹.

The lowest glyphosate low doses used by França et al. (2010) (57.6 g ha⁻¹) and Rusin et al. (2017) (407.5 g ha⁻¹) on young coffee and peach plants, respectively, showed no significant difference for stem diameter. This was probably because the low doses were high for a hormetic effect on stem diameter.

The glyphosate low doses of 22.5 and 45 g ha⁻¹ resulted in greater vegetative gains, different from the results of the others parameters evaluated, and the coffee plants presented higher number of lateral sprouts under the herbicide low dose

Table 6: Lorentz model for data of plant height, stem diameter, total leaf area and number of sprouts of coffee plants (*Coffea arabica* cv. Catucaí Vermelho 144), evaluated at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

	DAA	Ymin	A	X0	W	R ²	MAPE
Plant height	7	26.37474	528.6847	25.70564	159.365	0.1968	2.6499
	21	28.0001	430.4349	22.6009	45.7675	0.8539	2.3941
	35	28.1188	436.4349	22.9055	41.4766	0.9803	0.7079
	49	28.2342	492.2484	26.8183	59.8881	0.7336	2.6807
	63	31.1796	593.1951	26.9001	105.0158	0.9355	0.9456
Stem diameter	7	0.4021	0	25.2950	252.6650	0	3.7905
	21	0.4575	6.1960	22.586	42.8945	0.6294	3.6985
	35	0.4333	9.9519	22.5839	34.6293	0.9318	2.1207
	49	0.4288	11.0142	22.7535	34.4442	0.8957	3.8158
	63	0.5881	10.3885	16.8241	26.3080	0.7766	4.5246
Total leaf area	7	161.3388	54896.42	33.2282	0.4000	0.3049	8.267
	21	165.799	248720.6	31.1424	0.1570	0.3551	19.11
	35	138.7487	33047.71	0	108.4798	0.9371	7.2757
	49	103.5361	18430.38	19.6222	42.4007	0.9938	4.4611
	63	77.3060	11178.18	16.4064	38.0822	0.8712	24.0627
Number of sprouts	7	3.2672	22362.86	312.574	1.0927	0.5263	12.3151
	21	3.5417	0	26.2796	218.3161	0	10.80
	35	2.2702	29774.23	318.8123	1.4548	0.52	104.6586
	49	1.4553	52758.57	295.5173	2.5776	0.8568	42.5329
	63	2.0964	2461.718	356.3677	0.1203	0.8977	17.4682

of 360 g ha⁻¹. Further researches could evaluate the viability of increasing the number of lateral sprouts by using glyphosate after pruning the coffee plants, considering that increases in number of sprouts combined with an early and more vigorous sprouting could make the canopy reform faster. However, the viability of this technique should prove that the glyphosate applied after the pruning will result in gains in yield or in canopy reform quality for coffee crops.

However, when working with another species of coffee, such as *Coffea canephora*, the stimulation of sprouting can be an important strategy to increase the number of plant cuttings in clonal gardens and, consequently, increase the extension of

the seedling nursery of *Coffea canephora*, as the propagation of seedlings of this species is carried out, almost entirely, by cuttings.

According to Calabrese (2014) and Vargas-Hernandez et al. (2017), a strong management strategy for hormesis maintains an ecologically correct environment while resulting in a desirable agricultural yield.

The response of number of sprouts of coffee plants to hormetic effect was different from the other parameters and other results found in the literature (Brito et al., 2017; Carvalho et al., 2013; França et al., 2010; Schrubbers et al., 2014), but none of them reports hormetic effect using ¼ of the commercially recommended dose of glyphosate.

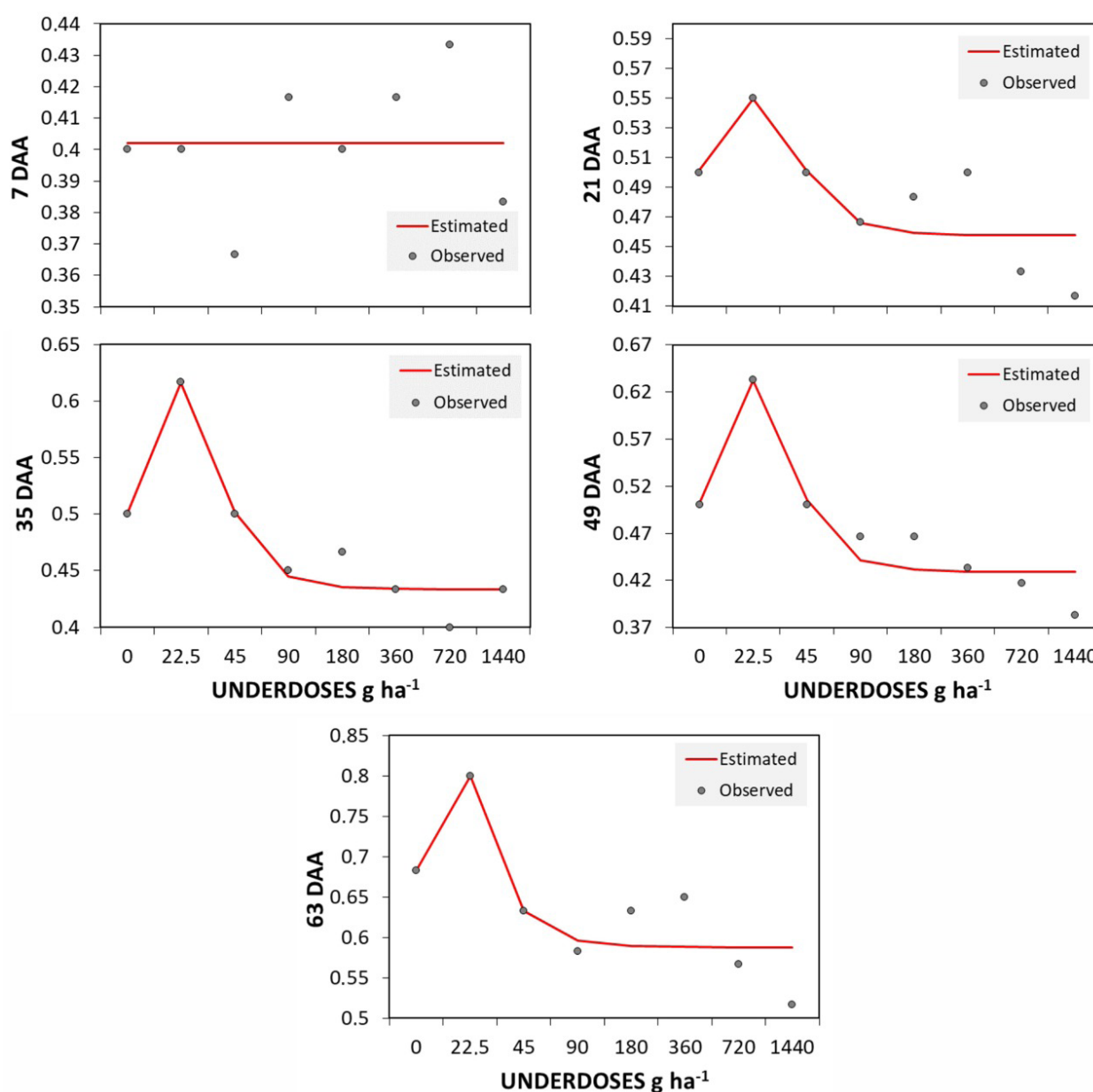


Figure 4: Lorentz model for data of stem diameter of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) evaluated at different days after application (DAA) of glyphosate herbicide at different doses (g ha⁻¹). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

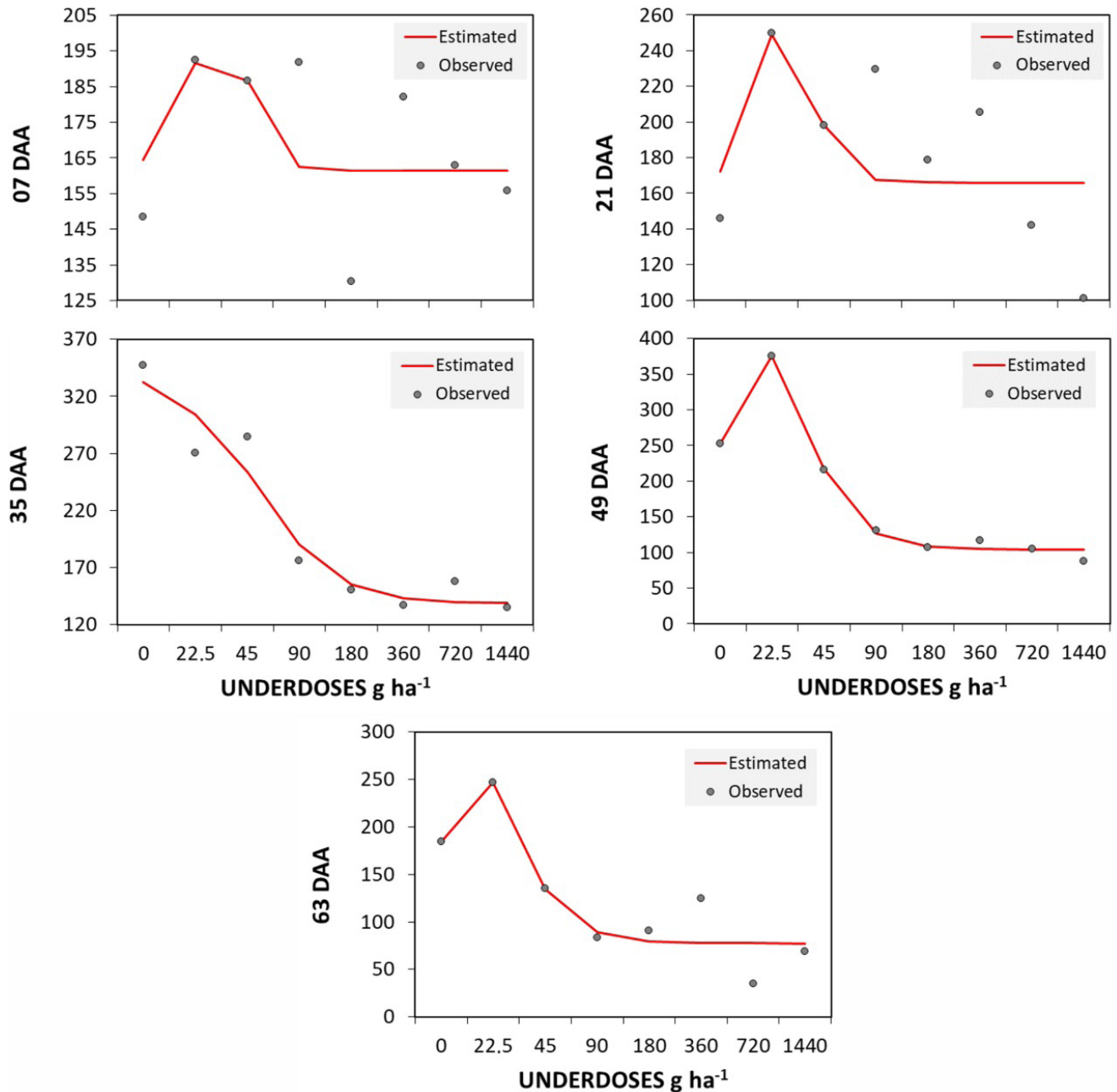


Figure 5: Lorentz model for data of total leaf area of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) evaluated at different days after application (DAA) of glyphosate herbicide at different doses (g ha^{-1}). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

One factor that also needs clarification is whether the use of glyphosate for hormetic effect on coffee crops is permanent throughout the development of the plants, i.e., whether the gains in dry weight will remain for a longer period throughout the plant life cycle. The results of the present work showed that the plant height of coffee plants was not affected at 49 DAA, presenting a slightly significant decrease when compared to that of plants of the control. Differently, the stem diameter was affected up to 63 DAA, and the leaf area presented hormetic effect only at 21 DAA.

Agathokleous, Kitao and Calabrese (2020) reported that the stimulation induced by chemical products is greater in a short period (1 month), reaching up to 150% higher values than the control, followed by decreases of 120% to 130%, which is maintained in the long-term.

Belz and Duke (2014) reported that the use of hormetic effect may be not practical because the transitory nature of effects and the potential effects on the progeny of the treated crop, mainly perennial crops.

Three different situations than that reported by Agathokleous, Kitao and Calabrese (2020) were found in the present work: the hormetic effect on plant height was practically cancelled at 49 DAA; the stem diameter values were high up to the end of the experiment, denoting that the study should be conducted in a longer time, until a better stability of this variable; and the total leaf area showed hormetic effect from 21 DAA.

Moreover, glyphosate has a slow effect inside the plant (Schrubbers et al., 2016), which may have delayed the hormetic effect on some characteristics.

Castanheira et al. (2019) tested application of several glyphosate low doses to coffee plants, whose lowest low dose was 144 g ha^{-1} , and found no effect on the physiology and leaf anatomy of coffee plants at 104 DAA.

A permanent hormetic effect on the development of seedlings in nurseries and in field crops, caused by application of low doses of glyphosate, may result in benefits, such as planting of more vigorous seedlings, improvement of survival of seedlings in the field when the herbicide low dose is applied to recently transplanted plants, increases in

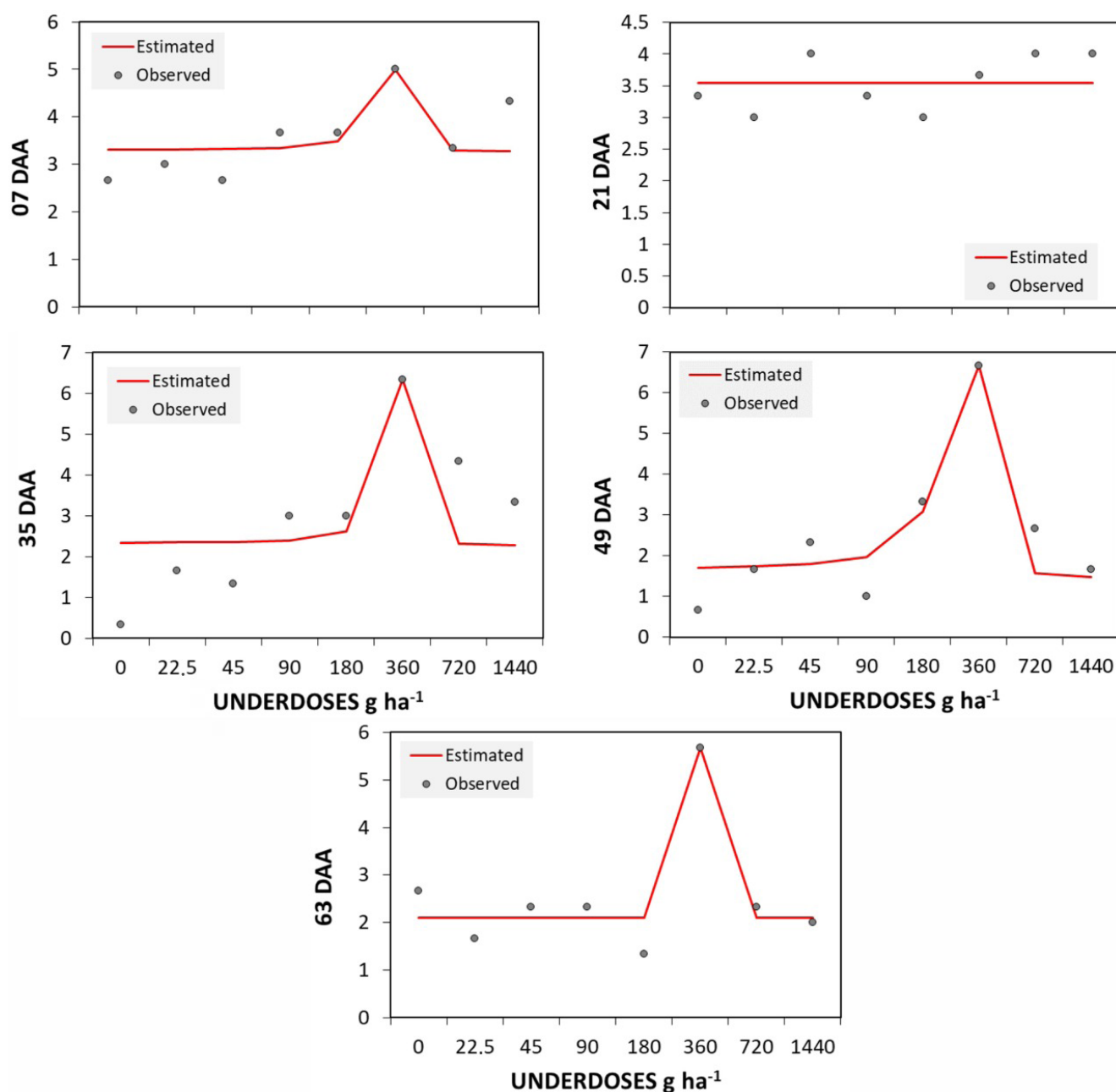


Figure 6: Lorentz model for data of number of sprouts of coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) evaluated at different days after application (DAA) of glyphosate herbicide at different doses (g ha^{-1}). Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

crop yield, and improvements of coffee quality by increasing sugar contents when applying the herbicide at the grain filling stage.

The lowest glyphosate doses tested (1.5% and 3% of the commercially recommended dose) was lower than that used

by Brito et al. (2017) (5%). The results of plant height, stem diameter, and total leaf area showed that the coffee crop is an exception for hormetic effect when using glyphosate, because greater hormetic effect could be found using glyphosate doses lower than those used in the present work.



Figure 7: Narrowing of leaves (A), healthy leaves (B), and overgrowth of orthotropic branches (C) in coffee plants subjected to glyphosate doses of 180 (left) and 360 (right) g ha⁻¹, evaluated at 63 days after application. Muzambinho, MG, Brazil. Agricultural year: 2018/2019.



Figure 8: Narrowing of leaves (A), darkening of old leaves (B), and overgrowth of orthotropic branches (C) of coffee plants subjected to glyphosate doses of 720 (left) and 1,440 (right) g ha⁻¹, evaluated at 63 days after application. Muzambinho, MG, Brazil. Agricultural year: 2018/2019.



Figure 9: Narrowing of leaves (A), shortening of internodes (B), and new healthy leaves (C) of coffee plants at 56 days after application of a glyphosate dose of 360 g ha⁻¹. Muzambinho, MG, Brazil. Agricultural year: 2018/2019.

The no-observed-adverse-effect level (NOAEL) is a dose that describes the x axis above 0 with no hormetic effect or toxicity. Thus, a precise identification of hormetic effect values requires the analysis of the low dose at the NOAEL, as shown by Agathokleous, Kitao and Calabrese (2020), who reported that pH affects the curve peak position in the x axis; a pH closer to neutrality enables the reduction of the hormetic low doses to values closer to 0.

The ideal hormetic effect for coffee plants is probably found with glyphosate doses lower than 22.5 g ha⁻¹, varying from 5.625 g ha⁻¹ to 11.25 g ha⁻¹. Moreover, understanding the hormetic effect requires analysis of plant height and total leaf area for longer periods.

Moreover, the tolerance of coffee genotypes and cultivars to glyphosate is different. França et al. (2010) found that the cultivar Topázio was more sensitive to glyphosate than Oeiras and Catucaí. Schrubbers et al. (2014) reported that the coffee cultivar Catuaí Vermelho IAC-144 is more tolerant to glyphosate than other varieties evaluated. Other studies also reported differences between coffee cultivars regarding tolerance to glyphosate (França et al., 2010; França et al., 2013; Carvalho et al., 2013).

The hormetic effect can be used to increase the production of several crops, but it requires to know whether the effect is permanent and cumulative and the best time to apply the glyphosate.

Silva et al. (2009) found stimuli for growth and initial development of sugarcane plants when using a sub-low dose of 1.8 g ha⁻¹, as also used by Pincelli-Souza et al. (2020), who found higher P absorption as an indicative of hormetic effect. Pincelli-Souza et al. (2020) found 38% increase in sugarcane yield and, when they evaluated application time of glyphosate low doses over the crop cycle, they found 47% increase in sugarcane yield by applying the herbicide at the beginning of the crop vegetative development.

The time for coffee plants to overcome the toxicity symptoms or for the glyphosate effects to decrease is also important. França et al. (2013) evaluated toxicity effects of glyphosate on coffee plants at 3, 10, 45, and 120 DAA and found higher toxicity for doses lower than 250 g ha⁻¹ up to 10 DAA and that this effect decreased over time; the effect of doses lower than 250 g ha⁻¹ decreased, and the effect of low doses higher than 250 g ha⁻¹ were still higher in the plants at 45 DAA.

Carvalho et al. (2013) found larger variation in metabolic activities of coffee plants (for all tested cultivars) at 15 DAA when compared to those at 45 DAA, confirming the results of França et al. (2013).

The plants were evaluated until they show recovery of the toxicity symptoms. However, destructive evaluations were used and the whole effect of the herbicide may have not been found due to the limited quantity of plants at the end of the experiment.

4.2 Lorentz model for the hormetic effect

The Lorentz model is used to identify peaks (Aparecido; Rolim, 2020) for hormetic effects situations, in which the herbicide effects gradually decrease as the herbicide dose is decreased, until the occurrence of an intense development (Cedergreen; Ritz; Treibig, 2005). Therefore, this model was applied to the studied variables.

When looking at Figure 2, it is clear that the recovery of the total leaf area was not noticeable, since the plants had a cumulative leaf deformation until little before this period, in which the development of wider, larger leaves begins, confirming the results of Castanheira et al. (2019), who noticed symptoms at approximately 60 DAA, but found no symptoms at 104 DAA, indicating that the plants recovery completely from the toxicity symptoms.

On the other hand, an assessment of NOAEL is required to improve the precision of the hormetic effect curve and the maximum point of this effect for this crop, which is probably below 22.5 g ha⁻¹ of glyphosate (Agathokleous; Kitao; Calabrese, 2020).

The estimated X0 value for number of sprouts was close to the low dose of 300 g ha⁻¹, little below the lowest dose tested (360 g ha⁻¹); this result, combined with the large variations of correlation factor and MAPE, denotes the need for studies on this characteristic to improve the precision of the glyphosate low dose that increases the number of sprouts of coffee plants.

The Ymin values for plant height and stem diameter were higher at 63 DAA (Table 6), denoting that the beginning of recovery from the glyphosate effects is at approximately 60 DAA, confirming the hypothesis of Schrubbers et al. (2016), who proposed that the glyphosate activity reduces at two months after the application.

Castanheira et al. (2019) found no symptoms of glyphosate toxicity in coffee plants at 104 DAA, thus, the glyphosate effect ceases between 2 and 3 months after application and the recovery of plants from toxicity starts before 60 DAA. Therefore, the total effect on plants subjected to high glyphosate low doses would not be longer than that for the low dose of 360 g ha⁻¹.

The correlation factor (R²) increased over the evaluations for all variables; the worse results were found at 7

DAA, which may be explained by a slow action of glyphosate on the plants (Schrubbers et al., 2016). Thus, glyphosate tends to stabilize inside of the plant (Schrubbers et al., 2016) and its effects tend to a stabilization over time, showing better results at 49 and 63 DAA.

The accuracy of the results (MAPE) for plant height, stem diameter, and total leaf area were mostly below 5%, denoting the confidence of the results obtained. However, MAPE presented no good results for number of sprouts, which had values always above 10%, denoting a need for more studies to confirm these results, although these results seem to be promising for the use of clonal gardens in *Coffea canephora* or recovery of crops under stress caused by pruning managements or weather factors, such as frosts and hail in *Coffea arabica*.

4.3 Symptoms of glyphosate toxicity in coffee plants

Schrubbers et al. (2014) described the width to length ratio of leaves to characterize the toxicity of glyphosate on coffee plants and determined the ratio of 0.41 as normal, 0.26 as light toxicity, and 0.19 as acute toxicity. However, coffee plants under higher glyphosate low doses have different symptoms than those under lower doses.

Schrubbers et al. (2014) also found darkening of old leaves as a symptom of glyphosate toxicity at higher low doses, which are close to the commercially recommended low dose. The symptoms of darkening of old leaves and narrowing of young leaves occurred at the same time in the present work. Castanheira et al. (2019) also found chlorosis and narrowing of young leaves of coffee plants after glyphosate applications.

The mortality of coffee plants showed that the glyphosate dose of 1,440 g ha⁻¹ did not kill all plants, but would justify their replacement by healthy plants in crops under formation for production. The remaining plants were evaluated for this dose. Schrubbers et al. (2014) found that coffee plants treated with 1,080 g ha⁻¹ were not killed, however, they were severely affected.

Schrubbers et al. (2016) found that coffee plants subjected to low glyphosate low doses accumulate greater proportion of the herbicide applied than those subjected to higher low doses. Thus, the glyphosate effect on coffee plants will probably end at similar times after application, regardless of the low dose used. However, no study analyzing the time to end the glyphosate effect on coffee plants is found.

Schrubbers et al. (2016) and Schrubbers et al. (2014) found a greater proportional accumulation of shikimic acid for low doses lower than those that they used; however, they found no significant difference between the treatments tested.

The end of the glyphosate effect on coffee plants is probably after the formation of 3 to 4 internodes, after

approximately 66 to 88 days, varying according to the season of the year. However, these data should be confirmed by new studies.

4.4 Use of glyphosate in coffee plants

Belz and Duke (2014) reported that the use of hormetic effect may be not practical because of the transitory nature of the effects and the potential effects in the progeny of the treated crop, mainly perennial crops. However, this transitional effect can bring some benefits to coffee crops, such as: the improvement of survival of seedlings in the field, when using scheduled applications of glyphosate low doses in the first months after planting; and probably some definitive gains in the crop formation, when more than one application of herbicide is done with a 2- or 3-month interval.

Researches should evaluate the viability of using glyphosate to increase the number of lateral sprouts, considering the data found in the present work. This information can be used for the recovery of crops under stress by pruning or weather factors, such as hails and frosts. The application of low doses of glyphosate may accelerate the recovery of crops subjected to pruning stress, minimizing losses.

Probably, the main result observed and with enormous practical potencial is the stimulation of lateral shoots of the orthotropic branch in the coffee tree. Although, this factor has been realized in *Coffea arabica*, this information is of enormous importance for the clonal gardens of *Coffea canephora* that use the development of clones to produce seedlings. Obviously, new studies should be carried out to improve this technique for both species, with analysis of shoot dry mass and number of orthotropic shoots.

Belz and Duke (2014) evaluated sugarcane crops and reported that the regrowth of perennials crops is impacted by exposure to low doses of herbicides from the last crop season. It was also found for the coffee plants evaluated in the present work.

Considering the production and yield of coffee plants, there is a potentially increase in sugars in plants subjected to glyphosate low doses, as already found for sugarcane (Pincelli-Souza et al., 2020; Silva et al., 2009). However, the use of hormetic effects in coffee crops should require more than one application of a low dose of glyphosate to improve the coffee quality, mainly from the flowering to grain filling stage.

5 CONCLUSIONS

The hormetic effect of glyphosate herbicide on coffee plants (*Coffea arabica* cv. Catuaí Vermelho 144) is found when using the low dose of 22.5 g ha⁻¹, mainly for plant height and stem diameter. The use of a low dose of 300 g ha⁻¹ results in hormetic effect for number of sprouts in orthotropic branches of coffee plants. The toxicity effects of glyphosate

to coffee plants last for approximately 2 months, when they start to recovery. The end of the glyphosate effects on coffee plants is visible when young leaves elongate and reach normal forms, which occurs at 56 days after application (DAA) of glyphosate.

Hormetic effect on coffee plants caused by application of glyphosate low doses is probably achieved when using low doses lower than 22.5 g ha⁻¹ for plant height and total leaf area.

Symptoms of leaf chlorosis and narrowing of leaves were found in coffee seedlings at 35 DAA.

The symptoms of toxicity of glyphosate found in the coffee plants were chlorosis, narrowing of young leaves, darkening of old leaves, and shortening of internodes.

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7 AUTHORS' CONTRIBUTION

GMoreiraTerra writing (course completion work) and data collection, GMaciêTerra data collect, LEOA Statistical analysis and graphing, CSA Data collection and laboratory conduction, KLBL-M Fundraising and laboratory management, GRBM Fundraising, data analysis, statistics and paper writing.

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