Low doses of glyphosate enhance growth, CO_2 assimilation, stomatal conductance and transpiration in sugarcane and eucalyptus

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Abstract

INTRODUCTION: Sublethal doses of herbicides can enhance plant growth and stimulate other process, an effect known as hormesis. The magnitude of hormesis is dependent on the plant species, the herbicide and its dose, plant development stage, and environmental parameters. Glyphosate hormesis is well established, but relatively little is known of the mechanism of this phenomenon. The objective of this study was to determine if low doses of glyphosate that cause growth stimulation in sugarcane and eucalyptus concomitantly stimulate CO_2 assimilation.

RESULTS: Shoot dry weight in both species increased at both 40 and 60 days after application of 6.2 to 20.2 g a.e. ha^{-1} glyphosate. The level of enhanced shoot dry weight was 11 to 37%, depending on the time after treatment and the species. Concomitantly, CO_2 assimilation, stomatal conductance, and transpiration were increased by glyphosate doses similar to those that caused growth increases.

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CONCLUSIONS: Glyphosate applied at low doses increased the dry weight of sugarcane and eucalyptus plants in all experiments. This hormetic effect was related to low dose effects on CO_2 assimilation rate, stomatal conductance and transpiration rate, indicating that low glyphosate doses enhance photosynthesis of plants.

Keywords: Hormesis, *Eucalyptus sp.*, *Saccharum* spp., low doses.

1 INTRODUCTION

Glyphosate is a systemic herbicide that inhibits the shikimic acid pathway enzyme 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS).[1,2] The shikimic acid pathway is responsible for the production of aromatic amino acids (tryptophan, phenylalanine and tyrosine) and many other phenolic compounds derived from these amino acids.[3-7]

Hormesis, the stimulatory effect of a subtoxic dose of a toxin,[8] is commonly found with herbicides.[9] In particular, there are numerous reports that low, subtoxic doses of glyphosate enhance plant growth and various physiological parameters.[9] In general, glyphosate hormesis is more reproducible and more pronounced than hormesis associated with other herbicides. For example, glyphosate applications between 1 and 10 g ae ha^{-1} can stimulate growth of eucalyptus (*Eucalyptis grandis*) from 40 to 100% depending on the plant part measured,[10] whereas hormetic effects of this magnitude are rare with other herbicides.[9]

Although utilizing glyphosate hormesis to improve yield is a daunting goal because of the unpredictability of the effect in the field, a better understanding of the mechanism of glyphosate-caused hormesis might improve the predictability of this phenomenon. The mechanism of glyphosate or any other herbicide-caused hormesis is not clearly understood, although, in the case of glyphosate-caused hormesis, it might be associated partly with the herbicide site of action,[11] since this effect is not observed in glyphosate-resistant plants at the

doses that cause hormesis in glyphosate-sensitive plants.[10] Low hormetic doses of glyphosate stimulated photosynthesis in barley plants.[11] The same authors proposed that at phytotoxic glyphosate doses, inhibition of photosynthesis is a secondary effect of inhibition of the shikimate pathway.

The objective of the present study was to determine the relationship between glyphosate hormesis, carbon assimilation, and transpiration in eucalyptus and sugarcane in an effort to further understand the mechanism of glyphosate hormesis in plants. We chose sugarcane and eucalyptus for these experiments because of our previous success in obtaining robust glyphosate-caused hormesis with these species.[10,12]

2 MATERIAL AND METHODS

Sugarcane stalks and eucalyptus seedlings were acquired in a commercial plant nursery. Two experiments were conducted in a greenhouse (air temperature between 27.0 ± 3.4 ˚C, with 65 ± 5% relative humidity, without supplementary lighting) with sugarcane (*Saccharum officinarum*, variety SP801842) and eucalyptus (*Eucalyptus urograndis,* clone 144). In the first experiment the plants were grown until 40 days after glyphosate application (DAA) and the other until 60 DAA. In the first experiment, chlorophyll content was determined at 15 and 30 DAA , CO_2 assimilation rate, stomatal conductance and transpiration rate were determined at 15 DAA, and dry matter was determined at the end of the experiment (40 DAA). In the second experiment, dry matter and glyphosate and shikimate content were determined 60 DAA. Both species were cultivated in 5-L pots filled with soil. Soil fertilization was made as indicated by the soil chemical analysis, following technical recommendations for these crops. Water was provided as required for normal growth and development of the plants.

2.1 Glyphosate treatment and growth measurments

Treatments consisted of 0, 1.8, 3.6, 7.2 18, 36, 72, 180, 360, 720 g a.e. ha^{-1} of glyphosate distributed in a complete randomized experimental design with five replications. A commercial glyphosate isopropylamine 480 g L^{-1} (360 g a.e. L^{-1}) SL (Roundup Original, Monsanto Company) was used. Application of the treatments was performed when the sugarcane plants had five to six true leaves and the eucalyptus shoots averaged 50 cm tall. A 1.5-m wide stationary boom sprayer was used with four XR 110.02 VS spray nozzles spaced every 0.5 m and positioned 0.5 m above the plants. The spray pressure adopted for the equipment was 2.0 kgf cm^{-2} with a speed of 3.6 km h^{-1} and spray volume of 200 L ha^{-1}.

For determination of dry weight, the leaves and stem were separated and then dried in an air-circulating oven at 40ºC. When the material reached a constant weight, it was weighed to 0.001 g precision on a Shimadzu scale (AY220).

2.2 Gas exchange methods

Assessments of gas exchange were carried out at 15 DAA in fully expanded leaves located on the middle third of the eucalyptus plants and and in the first completely expanded leaf with a visible auricle in sugarcane.[14] Analyses for doses above 180 g a.e. ha^{-1} in eucalyptus were not carried out due to the high level of toxicity caused by this dose of glyphosate. A photosynthesis open system instrument with a CO_2 and water vapor infrared analyzer (IRGA, model LI-6400, Li-Cor) was used. The differences between the CO_2 concentration and water vapor values present in the chamber with and without the samples allowed determination of CO_2 concentration and water vapor released (transpiration) and assimilated (CO_2 assimilation) via the stomata. Gas exchange measures consisted of the CO_2 assimilation rate (A, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol water vapor m^{-2} s^{-1}) and stomatal conductance (gs, mol m^{-2} s^{-1}). These variables were calculated by the data analysis software of the photosynthesis measuring equipment, which uses the general equation of gas exchanges. [15]

2.3 Glyphosate, aminomethylphosphonic acid (AMPA) and shikimate analysis

For the glyphosate, AMPA, shikimic acid analyses, oven-dried plant samples were crushed in a mortar with liquid nitrogen.[16] A 100-mg sample of the crushed matter was placed in a centrifuge tube with 10 mL of acidified water (pH 2.5). The tubes were then subjected to an ultrasonic bath at an ultrasonic frequency of 42 KHz for 30 min, and centrifuged at 4,000 *g* for 10 min at 20 ºC. The supernatant was collected and filtered in a Millex HV filter, 0.45 µm, with a 13-mm Durapore membrane and kept in an amber glass vial for further quantification of compounds by LC-MS/MS system using high performance liquid chromatography (HPLC) (Shimadzu, model Proeminence UFLC, equipped with two LC-20AD pumps, an SIL-20AC auto-injector, a DGU-20A5 degasser, CBM-20A system controller and CTO-20AC oven) and mass spectrometer, hybrid triple quadrupole (Triple Quad 4500, AB SCIEX).

2.3. Chlorophyll analyses

To determine the chlorophyll content, two leaves were collected, at 15 DAA and 30 DAA for the two species. The chlorophyll content determinations (mg g^{-1}) were based on methods and equations: [17] Chlorophyll a = (11.25 x A663 – 2.79 x A647); Chlorophyll b = (21.5 x A647 – 5.1 x A663); Carotenoids = (1000 x A470 – 1.82 x Chlorophyll a – 85.02 x Chlorophyll b)/ 190; where A is the absorbance at the wavelength indicated.

2.4 Statistical analysis

The data were submitted to analysis of variance by the F test and the means were compared by Tukey's test ($p \leq 0.05$). The statistical models used were those of Brain and Cousins[18] and Streibig,[19] as adapted by Velini et al.,[10] with the purpose of describing the dose-response curves with growth stimulation (Model 1) or without growth stimulation (Model 2) after application of glyphosate. The models were fitted by the SAS® software[20] (SAS, 2008), and the graphs were plotted by the SigmaPlot 12.5 software.

(Model 1) $y = F(x) = \frac{k+fx}{1+e^{bg}x^{b}} + d$

(Model 2) $y = F(x) = \frac{k}{1+e^{bg}x^{b}} + d$

where y = F (x) = treatment output; x = herbicide dosage; $k + d$ is the estimated F (x) for the control treatment (without herbicide application); d = estimated F (x) for herbicide dose causing maximum inhibition; b = determine the way in which F (x) decreases with dose; g = −ln (ED50) and ED50 is defined as the dose that gives 50% of the total achievable effect. The models used are different for only one additional coefficient, which multiplies the independent variable (x), allowing calculation of the increased sum of squares due to regression, including coefficient f with a value different from zero. Therefore, it is possible to test the increase in the model sum of squares resulting from the inclusion of the f constant, with a single degree of freedom. When the increase in the model sum of squares was significant, the hypothesis $f = 0$ was rejected, the occurrence of growth stimulation was accepted, then the complete model (Model 1) was used. [10] When the mean square value was not significant, the hypothesis $f = 0$ was accepted, and it was concluded that there was no growth stimulation at low doses, a standard sigmoid model (Model 2) was used for the data as by Velini, et al..[10]

For the variables glyphosate and shikimic acid concentrations, data were adjusted by linear regression (y = a + bx) as a function of the glyphosate dose, where y = treatment output; x = herbicide dose; a = intercept when x = 0 and b = slope.

3 RESULTS AND DISCUSSION

3.1 Effect on dry matter accumulation

Low doses of glyphosate produced an increase of dry weight of leaves, stems, and total shoots in both experiments with both plant species (Fig. 1 and 2, Tables 1 and 2). In the

first experiment, in which measurements were taken at 40 DAA (Fig. 1A and 2A, Tables 1 and 2), glyphosate doses of 7.8 and 7.4 g a.e. ha^{-1} caused increases of 28.8 and 35.3 % in total dry weight in sugarcane and eucalyptus, respectively. Whether considering leaf, stem or total shoot dry weight for sugarcane or eucalyptus, the range of glyphosate doses for the maximal hormetic amplitude was a narrow one of only ca. 5 to 9 g a.e. ha^{-1}. In the second experiment in which samples were taken at 60 DAA (Fig. 1B and 2B, Tables 1 and 2), glyphosate doses of at 14.5 and 19 and g a.e. ha^{-1} caused increases of 29.4 and 13.1% in total dry weight in sugarcane and eucalyptus, respectively. In the case of sugarcane, the hormetic effect on stem dry weight at 60 DAA (30.3% stimulation) contributed most to the total shoot effect, but this was not the case at 40 DAA. With eucalyptus, the hormetic effects on stem and leaves were similar. For sugarcane, the hormetic effect was higher at 60 than at 40 DAA. But, for eucalyptus, the effect was less at 60 than at 40 DAA.

Insert Figure 1

Insert Table 1

Insert Figure 2

Insert Table 2

Previous studies of glyphosate-caused hormesis with eucalyptus and sugarcane have given some similar results with respect to the hormetic dose ranges, but the amplitude of the effects have been different. In the study of Velini et al.[10] with eucalyptus at 60 DAA, the hormetic dose for dry weight, depending on the plant part, ranged from 2 to 4 g a.e. ha^{-1}, whereas, the leaf dry weight and stem dry weights were enhanced 60 and 53%, respectively. In this previous study, the root dry weight was enhanced 111% with 1.9 g a.e. ha^{-1} of glyphosate, and the net increase in dry weight of the entire plant was increased 69.8% with 2.6 g a.e. ha^{-1} of glyphosate. So, there was considerable net increase in dry weight as a result of treatment with a dose of glyphosate equivalent to less than 1% of the recommended dose

for weed management. Low glyphosate doses, between 3.6 and 7.2 g a.e. ha^{-1}, increase leaf dry weight of eucalyptus about 20%.[21] The dry weight of sugarcane shoots was increased about 35% at 21 DAA by 7.2 to 36 g a.e. ha^{-1} glyphosate, [14] whereas Silva et al.[22] found glyphosate at 1.8 g a.e. ha^{-1} to stimulate growth of sugarcane up to 85% for the dry mass of shoots. Our results are similar to these earlier findings.

Glyphosate hormesis has been observed in both weed and crop species with optimal hormetic doses usually varying from 1.8 to 36 g a.e. ha^{-1}.[9,10] The optimal dose of glyphosate for hormesis differs with plant species, time after treatment, what is measured, age and physiological status of the plants, and environmental factors.[9] For example, in a field study with *Brachiaria brizantha*, the optimal dosage for hormetic increases in biomass and plant height at 15 DAA was smaller than the optimal hormetic dose at 30 DAA.[23] With coffee plants,[24] an 18% increase in stalk diameter, 31% in leaf dry weight and 27% in total dry weight in experiments at 60 DAA with doses of glyphosate near 500 g a.e. ha^{-1}, but glyphosate hormesis was only found in plants treated 45 days after transplanting and not at 10 days after transplanting.

The duration of the hormetic effect after treatment is of great interest, because a long-lasting effect can result in yield increases. In greenhouse studies with barley plants, an increased growth rate in the first week after spraying with glyphosate doses below 60 g a.e. ha^{-1} was observed.[25] A hormetic effect was maintained for 6 weeks after spraying, but was lost after this time and did not result in a yield increase. With sugarcane and eucalyptus, we found that hormesis was maintained for more than 8 weeks and that in sugarcane, the effect was greater after 8 weeks than after 5-6 weeks (Table 1).

3.2 Effect of low glyphosate doses on gas exchange

Ultimately, most dry weight increase is due to carbon fixation which can be determined by infrared gas analysis of CO_2 uptake. In the first experiment, the gas exchange evaluation conducted 15 DAA in sugarcane plants had an increase of 99.4% in the CO_2 assimilation rate, compared to the control, for glyphosate applications at 6.1 g a.e. ha^{-1} (Fig. 3A, Table 3). With this dose, the CO_2 assimilation rate achieved maximum levels, of 19.4 μmol m^{-2} s^{-1}, almost twice that of the control, which was only 9.6 μmol m^{-2} s^{-1}. In eucalyptus plants, the CO_2 assimilation rate increased maximally with glyphosate applications at 11.6 g a.e. ha^{-1}, with an increase of 98.6% compared to the control (Fig. 3D, Table 3). The processes that would cause an increased photosynthetic activity in plants treated with low doses of glyphosate are not clearly understood. The stimulation of photosynthesis in barley plants was reported when exposed to low doses of glyphosate (11 to 45 g a.e. ha^{-1}), and this effect remained until harvest.[12] According to these authors, changes in carbon fixation rate, shikimic acid content and carbohydrate translocation may influence the occurrence of these phenomena.

Stomatal conductance assessed 15 DAA in eucalyptus leaves indicated an increase with application of low doses of glyphosate (Fig. 3E. Table 3). For doses at 11.4 g e.a ha^{-1}, stomatal conductance reached levels of 0.206 mol m^{-2} s^{-1}, corresponding to the 82.7% increase in relation to the control. Similarly, stomatal conductance in sugarcane increased a dose of 3.4 g a.e. ha^{-1}, (Fig. 3B, Table 3). The maximum stomatal conductance value observed was 0.123 mol m^{-2} s^{-1}, and this value corresponds to an increase of 82.7% with respect to the control.

The sugarcane transpiration rate increased maximally (80.6%) with glyphosate application at 3.4 g a.e. ha^{-1} compared to the control, reaching 2.3 mmol m^{-2} s^{-1} (Fig. 3C, Table 3). The transpiration rate of eucalyptus increased with glyphosate application (Fig. 3F,

Table 3) with a maximum value of 3.3 mmol m^{-2} s^{-1} (86.1% higher than the control) found at a glyphosate application rate of 11.2 g a.e ha^{-1} was 3.3 mmol m^{-2} s^{-1}.

Enter Figure 3

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The doses that caused increases in gas exchanges variables were all below 10 g a.e. ha^{-1}. In glyphosate-treated eucalyptus plants, CO_2 uptake, stomatal conductance and transpiration rate diminished when doses above 43.2 g a.e. ha^{-1} were applied.[26]

Stomatal movement is the main mechanism of control of gas exchange in all but primitive plants, because virtually all CO_2 influx and water efflux occurs through the stomata. Control of gas exchange is a complex process because the plants face the dilemma associated with the proper balance between CO_2 uptake and water loss by maintaining a stomatal aperture that avoids water stress while maximizing carbon fixation, a balance that is problematic under even mild water stress. Photosynthesis depends on flow of CO_2 into the cell, and this CO_2 flow depends on the stomatal opening.[27] Rubisco, the enzyme responsible for assimilation of CO_2, is a highly inefficient enzyme because O_2 competes with CO_2, resulting in photorespiration, a process that wastes ATP and NADPH from the light reactions of photosynthesis. Thus, the higher the CO_2 to O_2 ratio, the more efficiently Rubisco can function. Thus, increased stomatal conductance has a beneficial relation in the Rubisco activity and, consequently, in photosynthesis.[28] High rates of CO_2 uptake have a direct relationship with loss of water by transpiration. Thus, with adequate water, high stomatal conductance leads to high water consumption and a positive growth increase.[29]

Glyphosate was not detected in eucalyptus leaves with doses of 1.8 to 7.2 g a.e. ha^{-1} (Fig. 4B, Table 4) 1.8 to 36 g e.a ha^{-1} for sugarcane (Fig. 4A, Table 4). The minimum detection of glyphohsate in spiked untreated leaf tissue was 0.25 μg g dry wt^{-1}, with a recovery of about 85% for both species. The glyphosate levels found at the three highest rates

of glyphosate application were about ten-fold higher than those in sugarcane. AMPA was found only in eucalyptus and only at very low concentrations at the three highest glyphosate treatments. Considering that the herbicide was only quantified in the leaves, it is possible that a significant part of the herbicide had been translocated to the roots or stem by 60 DAA. With doses of 18 g a.e. ha^{-1} and higher, the glyphosate levels in the leaves increased progressively up to the dose of 360 g a.e. ha^{-1}, at which the glyphosate concentration for sugarcane was 0.35 μg g^{-1} dry weight and that of eucalyptus was 6.3 μg g^{-1} dry weight (Fig. 4).

Enter Figure 4.

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At 60 DAA of 360 g a.e. ha^{-1} glyphosate, the shikimic acid concentration in the sugarcane leaves increased 23.6% compared to the control (Fig. 4A), reaching a level of about 2.3 μg g^{-1} dry weight. In eucalyptus leaves, for the same dosage, the shikimic acid levels increased 2.5 times in relation to the control, reaching values of about 36.1 μg g^{-1} dry weight. Shikimic acid accumulation is usually directly associated with glyphosate concentrations.[30] The dose/response curves of the glyphosate and shikimate contents were similar in both species, although, the control levels of shikimate were higher in eucalyptus than for sugarcane.

The finding that no glyphosate or glyphosate-induced shikimate levels were found at the doses of glyphosate that stimulated growth is surprising. However, glyphosate and shikimate concentrations were determined at 60 DAA, and the carbon assimilation was measured at 15 DAA. Measureable glyphosate and an increase in shikimate could have been detected at 15 DAA and lost later due to translocation and/or degradation. Glyphosate-elevated shikimate levels are known be reduced or lost over time.[31]

An increase in the content of chlorophylls and carotenoids occurred in sugarcane plants that received low doses of glyphosate (Table 5, Figure 5). However, there was no effect of hormetic glyphosate doses on chlorophyll in eucalyptus (data not shown). . Many studies have reported reductions in chlorophyll content in plants treated with phytotoxic doses of glyphosate.[*e.g.,* 32-34] However, subtoxic dose effects of glyphosate on chlorophyll content in plants are not found in the literature.

Enter Figure 5.

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CONCLUSIONS

Application of low doses of glyphosate, in the range of 5.8 to 19 g a.e. ha^{-1}, increased leaf, stem and total dry weight in sugarcane and eucalyptus plants at 40 and 60 DAA. At 15 DAA, CO_2 assimilation, stomatal conduction, and transpiration rates were stimulated by sublethal doses, ranging from 3.4 to 11.6 g a.e. ha^{-1}. The hormetic effect on carbon assimilation is likely to be responsible for the later hormetic effect on dry weight increases. The dose/response curves for glyphosate shikimate content were similar for sugarcane and eucalyptus, with both chemicals only increasing at doses above about 100 g a.e. ha^{-1}.

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Table 1. Parameters for the regression equations and F values for leaf, stem and total dry weight of dry weight of sugarcane plants (40 and 60 days after application). Values represent regression parameters ± standard error. Curves from these data are provided in Figure 1.

	Dry weight (% of control)		
	40 days after treatment		
	Leaf	**Stem**	**Total**
Hypothesis $f \neq 0$	17.51**	13.31**	24.03**
Regression	44.90**	26.60**	48.31**
Model	Model 1	Model 1	Model 1
R^2	0.99	0.98	0.99
Constants			
g	-2.33 ±0.47	-2.42 ± 0.70	-2.37 ± 0.56
b	1.79 ±0.25	1.62 ± 0.26	1.68 ± 0.23
k	48.37 ±8.91	54.13 ± 15.33	51.49 ± 11.51
d	50.15 ±8.83	37.47 ± 13.56	43.22 ± 10.91
f	9.58 ±6.09	11.70 ± 9.97	10.85 ± 7.73
	60 days after treatment		
	Leaf	**Stem**	**Total**
Hypothesis $f \neq 0$	11.50**	4.33*	4.32*
Regression	102.20**	50.51**	68.42**
Model	Model 1	Model 1	Model 1
R^2	0.99	0.99	0.99
Constants			
g	-3.50 ±0.10	-3.75 ±0.29	-3.53 ±0.31
b	3.06 ±0.33	2.85 ±0.75	2.62 ±0.62
k	87.40 ±3.19	94.09 ±7.36	91.75 ±7.88
d	17.71 ±2.66	19.77 ±5.12	18.58 ±3.43
f	2.47 ±0.66	1.44 ±1.34	2.13 ±1.84

*P < 0.05; **P < 0.01; Model 1 Y=((k+f*x)/(1+(e^(b*g))*(x^b))+d.

Table 2. Parameters for the regression equations and F values for leaf. stem and total dry weight of eucalyptus plants (40 and 60 days after application). Values represent regression parameters ± standard error. Curves from these data are provided in Figure 2.

	Dry weight (% of control)		
	40 days after treatment		
	Leaf	**Stem**	**Total**
Hypothesis $f \neq 0$	8.63**	8.74**	4.55**
Regression	55.8**	7.53**	15.52**
Model	Model 1	Model 1	Model 1
R^2	0.99	0.99	0.99
Constants			
g	-1.59 ±0.73	1.59 ±0.80	-1.97 ±0.77
b	1.52 ±0.24	1.56 ±0.27	1.48 ±0.20
k	14.88 ±11.10	12.10 ±10.47	32.60 ±12.24
d	84.96 ± 10.88	86.05 ±13.42	68.41 ±13.68
f	18.93±13.60	15.27 ±12.35	14.16 ±0.20
	60 days after treatment		
	Leaf	**Stem**	**Total**
Hypothesis $f \neq 0$	5.85*	15.57**	4.75*
Regression	25.47**	5.54**	21.25**
Model	Model 1	Model 1	Model 1
R^2	0.99	0.99	0.99
Constants			
g	-3.80 ±0.58	-1.81 ± 0.47	-3.77 ±0.74
b	1.95 ±0.37	1.77 ± 0.30	1.83 ±0.36
k	58.15 ±5.90	-3.39 ± 1.28	48.89 ±6.16
d	42.39 ±5.73	102.19 ± 4.65	53.15 ±7.04
f	1.21 ±1.07	5.73 ± 2.43	1.28 ±1.13

ns = not significant; *P < 0.05; **P < 0.01; Model 1 Y=((k+f*x)/(1+(e^(b*g))*(x^b))+d.; Model 2 Y=((k)/(1+(e^(b*g))*(x^b))+d.

Table 3. Parameters for the regression equations and F values for CO_2 assimilation rate. stomatal conductance and transpiration rate in sugarcane and eucalyptus leaves 15 days after

application of glyphosate. Values represent regression parameters ± standard error. Dose/response curves from these data are plotted in Fig. 3.

	Dry weight (% of control)		
	Sugarcane		
	CO_2 assimilation	**Stomatal conductance**	**Transpiration rate**
Hypothesis $f \neq 0$	13.95**	6.98*	8.21*
Regression	25.27**	7.35*	6.91**
Model	Model 1	Model 1	Model 1
R^2	0.98	0.98	0.97
Constants			
g	-1.82 ±0.45	-1.35 ±0.58	-1.06 ±1.06
b	1.59 ±0.16	1.71 ±0.25	1.52 ±0.28
k	6.54 ±1.98	0.04 ±0.01	0.50 ±0.41
d	3.50 ±1.70	0.03 ±0.01	0.73 ±0.18
f	4.09 ±2.01	0.04 ±0.02	0.88 ±0.97
	Eucalyptus		
	CO_2 assimilation	**Stomatal conductance**	**Transpiration rate**
Hypothesis $f \neq 0$	15.28**	10.34**	14.74**
Regression	6.64**	4.11*	4.78*
Model	Model 1	Model 1	Model 1
R^2	0.98	0.99	0.99
Constants			
g	-2.79 ±0.32	-2.67 ±1.61	-2.63 ±0.03
b	3.47 ±1.85	9.38 ±1.77	9.97 ±1,69
k	0.34 ±0.51	0.008 ±0.008	-0.03 ±0.11
d	2.01 ±0.45	0.103 ±0.011	1.77 ±0.19
f	0.25 ±0.14	0.009 ±0.003	0.16 ±0.04

*P < 0.05; **P < 0.01; Model 1 Y= ((k+f*x)/(1+(e^(b*g))*(x^b))+d.

Table 4. Parameters for the regression equations and F values for glyphosate and shikimic acid in sugarcane and eucalyptus leaves 60 days after application of glyphosate. Values represent regression parameters ± standard error. These data are plotted in Figure 4.

	Concentration (μg g^{-1} of dry mass)	
	Sugarcane	
	Glyphosate	**Shikimic acid**
F-values	12.74**	6.06**
R^2	0.99	0.894
Constants		
a	0.0010 ±0.00003	0.0014 ±0.0004
b	-0.0100 ±0.0045	1.8187 ±0.0585
	Eucalyptus	
	Glyphosate	**Shikimic acid**
F-values	41.81**	3.08*
R^2	0.98	0.96
Constants		
a	0.018 ±0.001	0.062 ±0.005
b	-0.231 ±0.102	14.048 ±0.628

ns = not significant; *P < 0.05; **P < 0.01; y= ax+y0

Table 5. Parameters for the regression equations and F values for chlorophyll a. b and total in sugarcane leaves 15 and 30 days after application of glyphosate. Values represent regression parameters ± standard error. Dose/response curves from these data are plotted in Figure 5A.

	Dry weight (% of control)		
	Sugarcane 15 days after treatment		
	Chlorophyll *a*	**Chlorophyll *b***	**Chlorophyll *total***
Hypothesis $f \neq 0$	19.04**	90.48**	13.96**
Regression	96.70**	30.24**	33.10**
Model	Model 1	Model 1	Model 1
R^2	0.98	0.99	0.99
Constants			
g	-2.98 ±0.17	-4.75 ±0.25	-2.56 ±0.51
b	3.63 ±0.88	3.32 ±1.11	1.72 ±0.23
k	8.13 ±1.00	0.93 ±0.31	7.97 ±1.44
d	1.84 ±0.99	2.69 ±1.15	5.13 ±2.77
f	0.53 ±0.27	0.07 ±0.02	1.21 ±0.84
	Sugarcane 30 days after treatment		
	Chlorophyll *a*	**Chlorophyll *b***	**Chlorophyll *total***
Hypothesis $f \neq 0$	5.24*	7.15*	6.21*
Regression	74.99**	11.18**	22.68**
Model	Model 1	Model 1	Model 1
R^2	0.99	0.99	0.99
Constants			
g	-3.22 ±0.61	-2.63 ±0.41	-3.03 ±0.58
b	1.68 ±0.24	1.66 ±0.17	1.65 ±0.22
k	5.93 ±0.98	1.42 ± 0.24	7.58 ±1.31
d	4.39 ±2.15	1.92 ± 0.99	6.02 ±2.82
f	0.45± 0.37	0.25 ±0.13	0.73 ±0.56

*P < 0.05; **P < 0.01; Model 1 Y =((k+f*x)/(1+(e^(b*g))*(x^b))+d.

Figure 1. Leaf. stem and total dry weight of sugarcane plants 40 days (A) and 60 days (B) after application of glyphosate. Data and statistics for this figure are provided in Table 1.

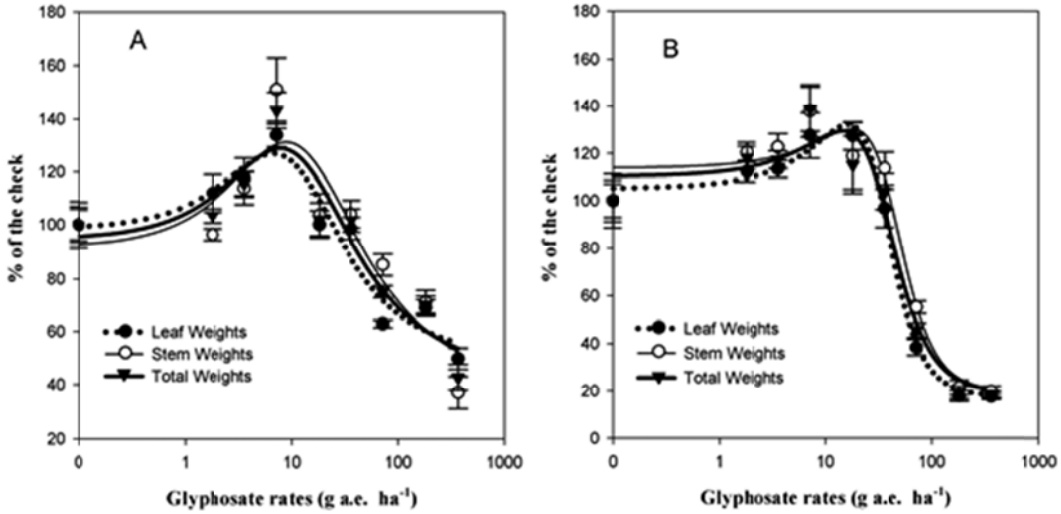

Figure 2. Leaf. stem and total dry weight of eucalyptus plants 40 days (A) and 60 days (B) after application of glyphosate. Data and statistics for this figure are provided in Table 2.

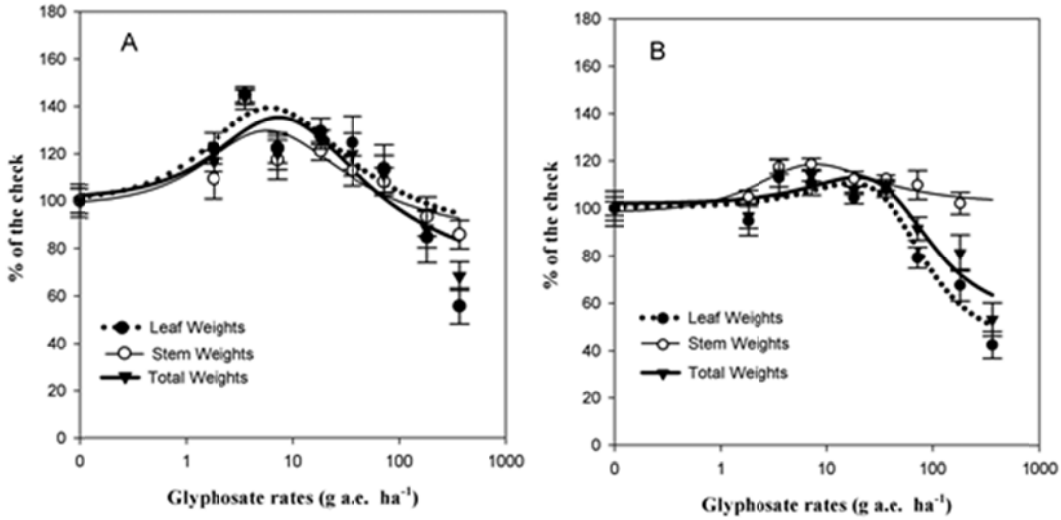

Figure 3. CO_2 assimilation rate (*A*. µmol m^{-2} s^{-1}) (A and D). stomatal conductance (*gs*. mol m^{-2} s^{-1}) (B and E) and transpiration rate (*E*. mmol m^{-2} s^{-1}) (C and F) in sugarcane leaves (left) and eucalyptus leaves (right) 15 days after application of glyphosate. Data and statistics for this figure are provided in Table 3.

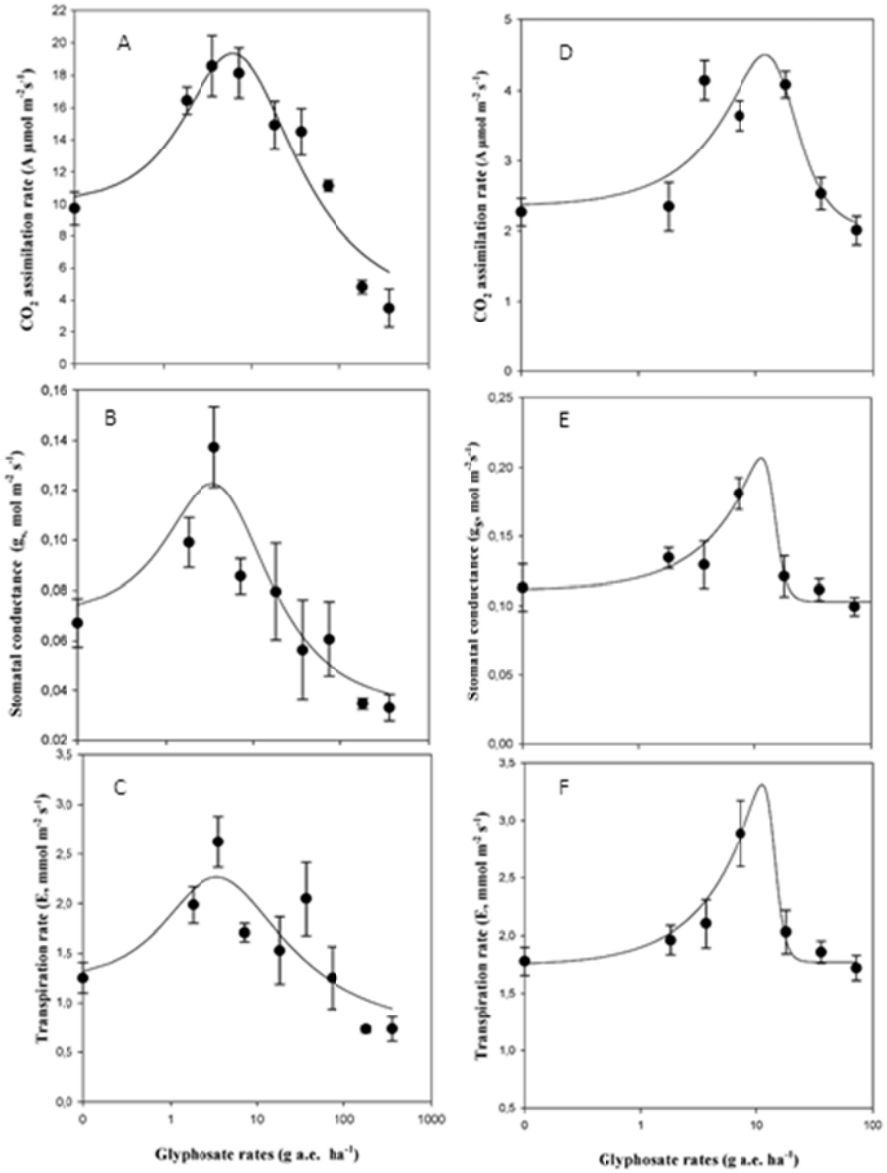

Figure 4. Glyphosate and shikimic acid in sugarcane (A) and eucalyptus (B) leaves 60 days after application of glyphosate. Data and statistics for this figure are provided in Table 4.

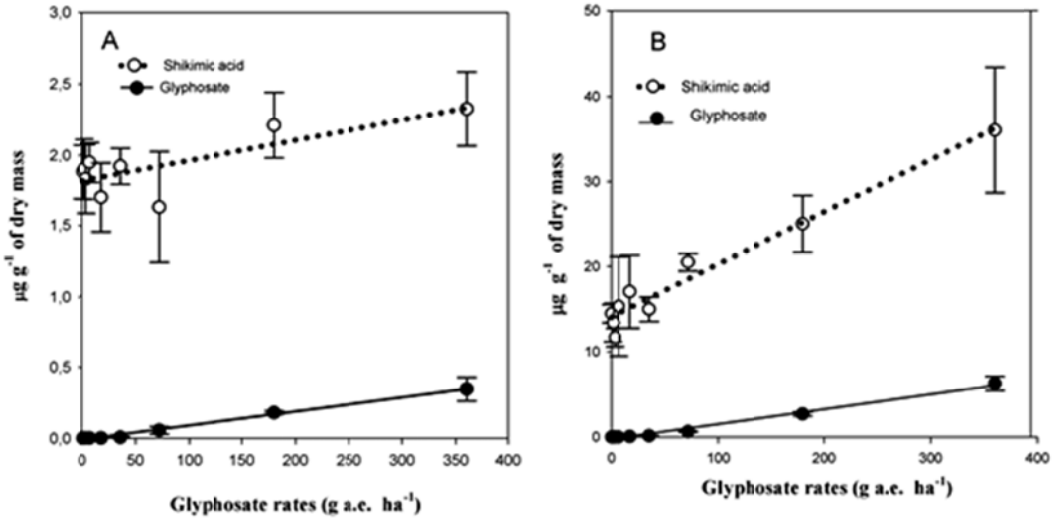

Figure 5. Chlorophyll *a. b* and *total* contents in sugarcane leaves 15 (A) and 30 (B) days after application of glyphosate. Data and statistics for this figure are provided in Table 5.