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Sugarcane's chemical ripeners: effects on growth and gas exchange of *Citharexylum myrianthum*, a Brazilian native tree species

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Abstract

Chemical ripeners are being used to anticipate the ripening process of sugarcane. These products act in specific enzyme or protein systems of plants, altering their functionality. However, these products can seriously affect the development of non-target species. In light of the proximity of permanent preservation areas to sugarcane plantations, we aimed at understanding the effect of simulated drift of chemical ripeners on 18-month age seedlings of *Citharexylum myrianthum*, a Brazilian native tree species. The experiment was conducted under semi-controlled conditions using a randomized block design with six replications. Treatments consisted of a 6x2 factorial arrangement, corresponding to six concentrations of glyphosate and sulfometuron-methyl, equivalent to 0, 5, 15, 30, 45, and 60% of commercial dose recommended for sugarcane. Plant height, stem diameter, number of leaves, total chlorophyll content, chlorophyll *a* fluorescence, and gas exchange were measured at 7, 30, and 60 days after application (DAA),. The leaf area and dry mass of plants were also measured at the end of the experimental period (60 DAA),. Both ripeners caused visual symptoms of phytointoxication in *C. myrianthum*, which were accompanied by alterations in gas exchange until 30 DAA. At 60 DAA, all plants treated with glyphosate recovered their photosynthetic capacity, without detrimental effect on their initial development, while those treated with 60% of sulfometuron-methyl showed limited growth in height coupled to super sprout due to the death of apical meristem. Despite no significant difference in total dry mass, the development of these plants was affected.

Keywords: Brazilian native species; drift; glyphosate; sulfometuron-methyl; reforestation.
Abbreviations: ALS_acetolactate-synthase; A_CO_2 assimilation rate; Chl_Total chlorophyll content; C_i_internal CO_2 concentration; DAA_Days after application; DAP_Days after planting; Diam_Stem diameter; DML_Dry mass of leaves; E_transpiration rate; F_v/F_m_Chlorophyll *a* fluorescence; g_s_stomatal conductance; PSII_Photosystem II.

Introduction

Despite techniques related to vegetal improvement representing the main means of increasing production, the expansion of agricultural boundaries continues to be used for this purpose (Nori et al., 2013). However, in the absence of environmental planning beforehand, this expansion causes forest fragmentation and loss of biodiversity (Potapov et al., 2017). Reductions in forests result in damage to the ecosystems and may compromise some essential processes to maintain the biological diversity of these areas, such as: stabilization of watercourse banks; the surface and subsurface filtration of water; and mitigation of sediment transport to waterways. As a result of the commitment of these processes, the local temperature of waters as well as their quality and quantity end up being altered, influencing all local fauna and flora (Mori et al., 2013). Consequently, increase in initiatives for restoring degraded areas has been observed in the last decades (Jellinek et al., 2013), mainly in the riparian areas of Brazilian states, basically due to two factors: raised awareness in society and legal requirements.
Thus, the tree species *Citharexylum myrianthum* Cham. (Verbenaceae) is highlighted, since it is widely used in areas of reforestation in these regions. This species originates from Dense Ombrophilous Forests – Atlantic Forest, and it is also found in riparian forests of the semideciduous seasonal forest (Lorenzi, 1992). This species is commonly observed in early stages of ecological succession of forests, and its fruits are appreciated by local fauna (Vasconcelo and Aguiar, 1982), becoming important component in the regeneration of degraded areas. Some of passive reforestation areas, especially riparian forests, are inside or close to properties in

which agricultural crops dominate, with sugarcane being the main emerging crop in various Brazilian states (Conab, 2017). Depending on the climate conditions for sugarcane maturation process, plant vegetative growth can be stimulated at the expense of sucrose accumulation, implying the production of lower quality material or even its scarcity (Viana et al., 2008). In order to bring forward the ripening process and help in planning sugarcane harvest, chemical ripeners have been used, many of which are herbicides applied in sub-doses that act in specific enzyme or protein systems of plants by altering their functionality (Alvarez et al., 2012). Among chemical products used as ripeners, glyphosate stands out, being widely used as growth inhibitor, as well as sulfometuron-methyl, which is a vegetal growth regulator (Correia and Leite, 2012).

The sugar alcohol sector has been seeking to improve its applications of phytosanitary products as a way of reducing spending, and with this aim, aerial applications are being widely used for spraying herbicides and ripeners. However, they may increase the risk of these products drifting into non-target species (Gelmini et al., 1988). By definition, drift is the displacement of the applied product out of the desired target, a phenomenon that can occur due to the wind action, runoff or even volatilization of product diluent (Andef, 2017). Possibly, because of drift, part of ripeners and herbicides applied to sugarcane crops can reach non-target plants in neighboring areas when products were not applied properly.

Thus, with the hypothesis that the glyphosate and sulfometuron-methyl drift may negatively affect the initial growth of *C. myrianthum*, the objective of this work was to evaluate the effects of simulated glyphosate and sulfometuron-methyl drift on the gas exchange and initial growth characteristics of *C. myrianthum*.

Results

Symptoms of glyphosate and sulfometuron-methyl application on Citharexylum myrianthum

At 7 days after the application (DAA), visual effects of phytointoxication caused by both products were observed. The glyphosate introduced chlorosis into the first pair of fully expanded leaves in all of the treatments with more than 30% of commercial concentration (Figure 1). Regarding sulfometuron-methyl drift, the initial leaf coloring changed to yellow-bronze with 5% of commercial concentration upwards (Figure 2).

Citharexylum myrianthum biometric characteristics after application of chemicals

At the end of the experimental period, with the exception of the dry mass of leaves (DML), there was no significant difference between the treatments that received the application of glyphosate for any of the biometric characteristics assessed (Table 1). For DML, 15% and 30% concentration treatments were the ones with the highest values, statistically differing from the 45% treatment, which presented a 32% lower DML value than the 30% treatment; however, it should be noted that this difference was not reflected in the total dry mass of the plant (Table 1).

For the sulfometuron-methyl, as the concentration applied increased, there was increased number of leaves per plant, resulting from the loss in apical dominance. The plants treated with 60% of the commercial concentration presented death of the apical meristem (Figure 3-F) resulting in 287.8% more leaves than the control (0%), which obtained the lowest value, together with the 5% concentration (Table 1). In spite of this, the leaf area was not altered between the treatments, indicating super sprout (Figure 3-A and 3-E). The 5% treatment was the one with the greatest height, significantly differing from the 15% and 60% concentrations, which caused reductions of 23.5% and 30.9%, respectively. The 15% treatment was also the one that resulted in the smallest stem diameter, with a difference for the 5% and 0% concentrations. For the dry mass of plants, no significant difference was found between the concentrations tested (Table 1).

Citharexylum myrianthum photosynthetic characteristics

Plants that received 15% glyphosate application showed higher F_v/F_m values, differing from the highest concentrations but not from the lowest ones (Table 2). For the net CO_2 assimilation rate, the effect was the opposite, in that the 30% and 45% doses resulted in greater carbon sequestration in relation to the two lowest doses tested, differing statistically. Also, the 60% treatment caused a 39.3% reduction in stomatal conductance compared to the 30% treatment, which resulted in a higher value for the characteristic and differed from all of the treatments except the 45% one. For internal carbon, the 5% treatment resulted in the lowest value, only equaling the 60% treatment. The total chlorophyll content and transpiration rate did not present any significant difference between the treatments (Table 2).

Still at 15 DAA, plants that received the sulfometuron-methyl treatment in any concentration showed reductions in F_v/F_m, but not in total chlorophyll content (Table 2). In relation to the net assimilation rate, plants that received 5% of this product differed significantly from treatments with the three highest concentrations, with a 59% higher value than the plants that received 60% of the ripener concentration. For the other characteristics of gas exchange (stomatal conductance, internal carbon, and transpiration rate), all treatments showed pattern of results similar to the control, in which values were highest and differed from the treatments that received the 15% concentrations or more of sulfometuron-methyl, presenting reduction in the parameters indicated (Table 2).

From 30 DAA onwards, there was no difference between the treatments, independent of the product applied, both for F_v/F_m and for total chlorophyll content (Table 3). Regarding the gas exchange there was no difference in the CO_2 sequestration rate for glyphosate treatments. However, this product resulted in gains of up to 38.8% for transpiration in relation to the control in concentrations equal to or higher than 15% (Table 3). For stomatal conductance, only the 30% and 60% treatments differed statistically from the control, which presented the lowest level for this variable. For internal CO_2 concentration, again the plants that were treated with a 30% concentration of the product obtained the highest values, with significant

Table 1. Effect of different concentrations of glyphosate (Glizmax® 192 g e.a. ha^{-1}) and sulfometuron-methyl (Curavial® 15 g e.a. ha^{-1}) to *Citharexylum myrianthum* on plant height (cm), stem diameter (Diam – mm), number of leaves (N. Leaves), leaf area (L. Area – cm^2), dry mass of leaves (DMLeaf – g), dry mass of the stem (DMStem – g), and total dry mass (DMTotal – g), assessed at 60 days after spraying ripeners.

	Glyphosate						
Conc.	Diam	Height	N. Leaves	L. Area	DMLeaf	DMStem	DMTotal
0%	8.88	35.0	16.4	594.4	6.31 ab	6.82	13.1
5%	8.78	38.2	18.2	596.0	6.17 ab	7.40	13.5
15%	8.25	40.0	16.6	676.5	6.91 a	7.12	14.0
30%	9.42	39.5	15.8	692.3	7.36 a	7.46	14.8
45%	8.23	38.3	20.1	539.4	5.00 b	6.67	12.1
60%	8.82	33.6	21.6	564.7	6.08 ab	6.59	13.2
F (Conc.)	1.57^{ns}	2.27^{ns}	1.28^{ns}	2.10^{ns}	4.11**	1.20^{ns}	2.34^{ns}
CV (%)	8.56	11.5	17.4	13.5	15.3	14.5	12.2
	Sulfometuron-methyl						
Conc.	Diam	Height	N. Leaves	L. Area	DMLeaf	DMStem	DMTotal
0%	8.88 a	35.0 ab	16.4 d	594.4	6.31	6.82	13.1
5%	8.90 a	40.4 a	23.8 d	607.0	5.73	6.07	11.8
15%	7.58 b	30.9 bc	42.1 c	609.9	6.25	6.54	12.7
30%	8.38 ab	37.6 ab	42.6 c	688.4	6.43	6.31	12.7
45%	8.78 ab	35.2 ab	51.7 b	654.5	7.04	6.84	13.8
60%	7.76 ab	27.9 c	63.6 a	673.6	6.67	5.62	12.3
F (Conc.)	3.77**	7.11**	71.0**	2.10^{ns}	1.22^{ns}	1.20^{ns}	2.34^{ns}
CV (%)	8.56	11.5	17.4	13.5	15.3	14.5	12.2

Means followed by the same letter (column) do not differ from each other by the Tukey test at 5% of probability. ** = significant value at 1% of probability by the F test. ^{ns} = value not significant at 5% of probability by the F test. CV = Coefficient of variation. Conc. = Ripener concentration.

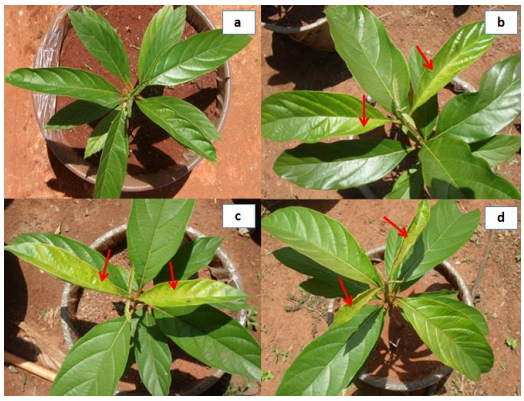

Fig 1. *Citharexylum myrianthum*, at 7 days after the application, with chlorosis (arrows) in the first pair of totally expanded leaves, caused by the drift of 30% (b), 45% (c), and 60% (d) of the commercial concentration of glyphosate (Glizmax® 192 g e.a. ha^{-1}), compared to the control treatment (a).

Table 2. Effect of different concentrations of glyphosate (Glizmax® 192 g e.a. ha^{-1}) and sulfometuron-methyl (Curavial® 15 g e.a. ha^{-1}) to *Citharexylum myrianthum* on chlorophyll *a* fluorescence (F_v/F_m), total relative chlorophyll content (Chlorphyll – UR), net CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-2}), stomatal conductance (g_s - mol H_2O m^{-2} s^{-2}), internal CO_2 concentration (C_i - µmol CO_2 mol^{-1}), and transpiration rate (E - mmol H_2O m^{-2} s^{-2}), assessed at 07 days after spraying ripeners.

	Glyphosate					
Conc.	F_v/F_m (07 DAA)	Chlorophyll (07 DAA)	A (07 DAA)	g_s (07 DAA)	C_i (07 DAA)	E (07 DAA)
0%	0.760 ab	48.7	8.11 b	0.256 bc	263.2 a	2.99
5%	0.755 ab	55.0	8.29 b	0.223 cd	241.8 b	2.87
15%	0.768 a	53.4	9.08 ab	0.255 bc	263.3 a	3.21
30%	0.712 bc	53.5	9.75 a	0.310 a	276.0 a	3.36
45%	0.690 c	53.5	9.73 a	0.280 ab	274.4 a	3.20
60%	0.696 c	53.5	8.92 ab	0.188 d	260.7 ab	3.01
F (Conc.)	8.77**	0.74^{ns}	6.06**	11.4**	6.19**	1.04^{ns}
CV (%)	4.01	8.23	8.55	14.9	4.79	17.5
	Sulfometuron-methyl					
Conc.	F_v/F_m (07 DAA)	Chlorophyll (07 DAA)	A (07 DAA)	g_s (07 DAA)	C_i (07 DAA)	E (07 DAA)
0%	0.760 a	48.7	8.11 ab	0.256 a	263.2 a	2.99 a
5%	0.698 b	56.0	8.32 a	0.187 b	253.5 ab	2.30 ab
15%	0.695 b	51.8	8.18 ab	0.145 bc	236.0 bc	1.74 bc
30%	0.686 b	51.7	7.07 bc	0.139 bc	232.7 c	1.33 c
45%	0.682 b	52.2	6.15 cd	0.122 c	231.2 c	1.37 c
60%	0.685 b	57.3	5.23 d	0.113 c	230.4 c	1.20 c
F (Conc.)	6.39**	0.74^{ns}	20.2**	18.0**	7.81**	15.5**
CV (%)	4.01	8.23	8.55	14.9	4.79	17.5

Means followed by the same letter (column) do not differ from each other by the Tukey test at 5% of probability. ** = significant value at 1% of probability by the F test. ^{ns} = value not significant at 5% of probability by the F test. CV = Coefficient of variation. DAA = Days after the application. Conc. = Ripener concentration.

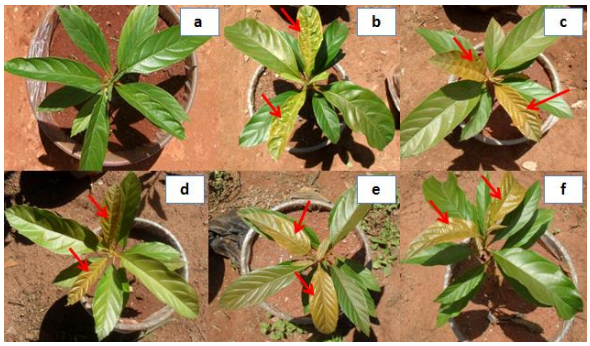

Fig 2. *Citharexylum myrianthum*, at 7 days after the application, with yellow-bronze coloring (arrows) in the first pair of totally expanded leaves, caused by the drift of 5% (b), 15% (c), 30% (d), 45% (e), and 60% (f) of the commercial concentration of sulfometuron-methyl (Curavial® 15 g e.a. ha^{-1}), compared to the control treatment (a).

Table 3. Effect of different concentrations of glyphosate (Glizmax® 192 g e.a. ha^{-1}) and sulfometuron-methyl (Curavial® 15 g e.a. ha^{-1}) to *Citharexylum myrianthum* on chlorophyll *a* fluorescence (F_v/F_m), total relative chlorophyll content (Chlorphyll – UR), net assimilation rate (A - µmol CO_2 m^{-2} s^{-2}), stomatal conductance (g_s - mol H_2O m^{-2} s^{-2}), internal CO_2 concentration (C_i - µmol CO_2 mol^{-1}), and transpiration rate (E - mmol H_2O m^{-2} s^{-2}), assessed at 30 days after spraying ripeners.

	Glyphosate					
Conc.	F_v/F_m (30 DAA)	Chlorophyll (30 DAA)	A (30 DAA)	g_s (30 DAA)	C_i (30 DAA)	E (30 DAA)
0%	0.776	47.2	9.02	0.265 c	266.5 abc	2.60 c
5%	0.783	48.5	9.16	0.288 bc	257.5 c	2.97 bc
15%	0.780	50.7	9.54	0.300 abc	272.7 abc	3.50 a
30%	0.787	50.8	9.67	0.354 a	280.1 a	3.61 a
45%	0.758	53.1	9.88	0.318 abc	277.1 ab	3.47 ab
60%	0.748	53.5	9.18	0.328 ab	263.0 bc	3.31 ab
F (Conc.)	0.80^{ns}	1.90^{ns}	1.88^{ns}	4.29**	4.82**	10.1**
CV (%)	4.56	6.63	6.88	13.6	3.63	10.3
	Sulfometuron-methyl					
Conc.	F_v/F_m (30 DAA)	Chlorophyll (30 DAA)	A (30 DAA)	g_s (30 DAA)	C_i (30 DAA)	E (30 DAA)
0%	0.766	47.2	9.02 a	0.265 a	266.5 a	2.60 ab
5%	0.784	56.4	8.72 a	0.253 ab	259.0 a	2.70 a
15%	0.786	56.9	8.97 a	0.233 ab	260.1 a	2.64 ab
30%	0.791	56.8	8.53 a	0.246 ab	264.7 a	2.61 ab
45%	0.792	56.6	7.30 b	0.196 b	264.3 a	2.28 ab
60%	0.790	59.6	6.69 b	0.190 b	242.1 b	2.14 b
F (Conc.)	0.80^{ns}	1.90^{ns}	15.5**	4.14**	5.19**	3.54**
CV (%)	4.56	6.63	6.88	13.6	3.63	10.3

Means followed by the same letter (column) do not differ from each other by the Tukey test at 5% of probability. ** = significant value at 1% of probability by the F test. ^{ns} = value not significant at 5% of probability by the F test. CV = Coefficient of variation. DAA = Days after the application. Conc. = Ripener concentration.

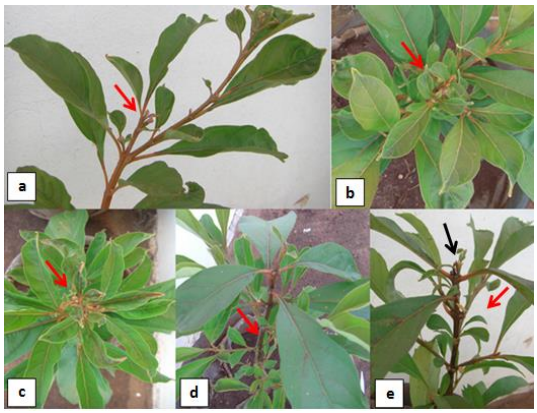

Fig 3. Detail of the visual symptoms of phytointoxication at 60 days after the application of sulfometuron-methyl in *Citharexylum myrianthum* plants treated with 5% (a), 15% (b), 30% (c) 45% (d), and 60% (e) of the commercial dose of sulfometuron-methyl (Curavial® 15 g e.a. ha^{-1}). Super sprout is observed (red arrow) in all of the treatments and death of the apical meristem (black arrow) for the highest concentration (e).

Table 4. Effect of different concentrations of glyphosate (Glizmax® 192 g e.a. ha^{-1}) and sulfometuron-methyl (Curavial® 15 g e.a. ha^{-1}) to *Citharexylum myrianthum* on chlorophyll *a* fluorescence (F_v/F_m), total relative chlorophyll content (Chlorphyll – UR), net CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-2}), stomatal conductance (g_s - mol H_2O m^{-2} s^{-2}), internal CO_2 concentration (C_i - µmol CO_2 mol^{-1}), and transpiration rate (E - mmol H_2O m^{-2} s^{-2}), assessed at 60 days after spraying ripeners.

	Glyphosate					
Conc.	F_v/F_m (60 DAA)	Chlorophyll (60 DAA)	A (60 DAA)	g_s (60 DAA)	C_i (60 DAA)	E (60 DAA)
0%	0.768	45.6	9.70	0.350	276.1	3.35
5%	0.789	44.9	9.44	0.320	270.3	3.16
15%	0.765	47.2	9.85	0.380	280.5	3.59
30%	0.747	47.6	9.91	0.395	283.5	3.63
45%	0.771	47.7	9.87	0.363	278.6	3.60
60%	0.766	47.3	9.34	0.351	273.8	3.35
F (Conc.)	1.49^{ns}	1.99^{ns}	0.12^{ns}	0.71^{ns}	0.23^{ns}	0.61^{ns}
CV (%)	3.68	8.30	9.12	19.0	4.17	11.5
	Sulfometuron-methyl					
Conc.	F_v/F_m (60 DAA)	Chlorophyll (60 DAA)	A (60 DAA)	g_s (60 DAA)	C_i (60 DAA)	E (60 DAA)
0%	0.768	45.6	9.70	0.350	276.1	3.35
5%	0.787	53.1	9.30	0.336	265.2	3.19
15%	0.773	55.3	9.66	0.315	276.5	3.22
30%	0.799	50.0	9.65	0.336	279.1	3.31
45%	0.784	50.1	9.75	0.328	281.8	3.61
60%	0.773	51.3	8.82	0.318	270.3	3.18
F (Conc.)	1.49^{ns}	1.99^{ns}	0.12^{ns}	0.71^{ns}	0.23^{ns}	0.61^{ns}
CV (%)	3.68	8.30	9.12	19.0	4.17	11.5

Means followed by the same letter (column) do not differ from each other by the Tukey test at 5% of probability. ^{ns} = value not significant at 5% of probability by the F test. CV = Coefficient of variation. DAA = Days after the application. Conc. = Ripener concentration.

difference for 60% and 5% doses, and plants treated with 5% presenting the lowest concentrations of internal carbon in the leaves (Tables 3).

In the net assimilation rate, only the two highest concentrations of sulfometuron-methyl caused reduction (22.3%) (Table 3). For stomatal conductance, again the only two treatments that differed from the control were 45% and 60%, with only the 60% treatment causing reduction in the concentration of internal carbon compared to the other treatments. Regarding transpiration rate, plants from the 5% treatment showed 26.1% higher value than the 60% treatment (Table 3).

In the evaluation for gas exchanges, quantum efficiency of photosystem II, and total chlorophyll content, carried out at the end of the experimental period (60 DAA), it was possible to observe total recovery of plants' photosynthetic apparatus, independent of which product was applied, with no significant difference being found for the characteristics evaluated (Table 4).

Discussion

Despite not carrying out any evaluation of phytotoxicity scores, *C. myrianthum* plants showed symptoms of chlorisis in the first expanded leaves of plants treated with the 30% to 60% concentrations at 7 DAA, with severity of the symptom being proportional to the increase in concentration. Similar symptoms were also observed in other tree species for concentrations equal to or higher than 86.4 g e.a. ha^{-1}, such as *Eucalyptus urophylla* and *E. grandis*, (Tuffi Santos et al., 2006), *Genipa americana* (Gusmão et al., 2011), *Aspidosperma desmanthum* (Rondon Neto et al., 2011), *Hevea brasiliensis* (Farias et al., 2012), and also in monocotyledon plants such as sorghum and corn for concentrations of 172 g e.a. ha^{-1} (Magalhães et al., 2001 a, b). Such damage is caused by the degradation of chloroplasts and also by inhibition of chloropyll formation, a common result observed in plants treated with glyphosate (Lee, 1981).

According to Vidal (1997), glyphosate can affect chlorophyll, since this molecule combines to the EPSP-S3P enzymatic complex in the chloroplast, inhibiting the synthesis of essential amino acids. This herbicide also causes indirect effects such as reducing RuBisCo enzymatic activity (Ahsan et al., 2008) and also disorganizing the photosynthetic apparatus (grain and intergrain), depending on the concentration used (María et al., 2005). This may have caused, for example, low values for quantum efficiency of photosystem II (PSII) in the evaluation carried out at 07 DAA (Table 2) in the plants that received the two highest concentrations tested. Thus, it is worth mentioning that the F_v/F_m fluorescence ratio is important physiological characteristic used in studies related to the different types of stresses as it is indicator of plants' photosynthetic capacity (Krause and Weis, 1991).

Variations in physiological responses were also observed by Pereira et al. (2010) at 7 days after spraying different concentrations of glyphosate to *E. grandis*. On this occasion, the authors found 22% reduction in transpiration and 18% reduction in the stomatal conductance of eucalyptus when 120 g e.a. ha^{-1} of the Scout® commercial formula was sprayed. However, despite there also being variation in physiological responses in this study at 07 DAA (Table 2), none of concentrations tested was enough to negatively affect the biometric characteristics of *C. myrianthum***,** since no treatment differed from the control (Table 1). This is probably due to the fact that plants' photosynthetic apparatus completely recovered within 60 days after application, as shown in Table 4. Velini et al. (2008) observed positive effect of low concentrations of glyphosate in some tree species, with 68% increase in total dry mass in *E. grandis* and 22% increase in *Pinus caribea*.

The positive effect resulting from low concentrations of originally toxic compounds is characterized as "hormesis" (Belz and Duke, 2014) and was also observed in other tree species and with other chemical products (Velini et al., 2008; Pereira et al., 2013; Pires et al., 2013; Correia and Villela, 2015). However, Belz and Duke (2014) note that the

observation or not of a hormetic effect is directly related with climate conditions under which the experiment is conducted, the species or clone used, the stage of plant development, and also the period between the product application and the final evaluation carried out; thus explaining the fact that no positive effect has been observed of sub-concentrations of glyphosate on the initial development of *C. myrianthum*.

For the sulfometuron-methyl, visual symptoms of phytointoxication were also observed at 7 DAA, with all of the concentrations applied causing yellow-bronze coloring in the first totally expanded leaves. From 15% concentrations onwards there was a loss of apical dominance (due to the death of apical meristem) and lateral leaf growth, and the higher the concentration, the greater the number of leaves (Table 1). As a result, plants grew less in height. Similar phytotoxic effects were also reported for *E. urograndis* (Correia and Villela, 2015).

This response may be related to this ripener's mode of action, which is inhibitor of ALS (acetolactate-synthase), belongs to the sulfonylurea chemical group and affects the synthesis of some essential amino acids (Cox, 2002; Zhou et al., 2007). As observed by Meschede et al. (2011), applying this product can interfere with photosynthesis by reducing the amount of carotenoids in leaves. This effect was also observed in this study, in which all of the applied concentrations differed from the control for quantum efficiency of the PSII at 07 DAA, and the two highest concentrations differed from the control in the gas exchange evaluations on the same date (Table 2).

Maxwell and Johnson (2000) note that normal values for quantum efficiency of PSII can vary between 0.850 and 0.750. Therefore, values within this range prove the efficiency of luminous energy capture by the reaction centers of this photosystem, which will result in the transport of electrons via PSII (Krause and Weis, 1991). Thus, values below this range of variation are taken as indications of stress, causing reductions in the plant's photosynthetic potential.

Despite quantum efficiency of PSII recovering in the subsequent evaluations and the fact that chlorophyll is greater in plants treated with sulfometuron-methyl in any concentration at 15 DAA, it is possible to note that at 30 DAA there is still not total recovery of photosynthetic apparatus of *C. myrianthum*, since the two highest concentrations presented lower values for net CO_2 assimilation rate, stomatal conductance, and internal carbon for highest concentration (Table 3).Thus, this phenomenon may also have caused the shorter height observed at the end of the experimental period for the 60% treatment (Table 1). Moreover, despite the sulfometuron-methyl not having directly affected the total dry mass of *C. myrianthum* plants, they presented super sprout, which combined to delayed growth, in relation to the control, represent morphological alterations that can impair development (Table 1).

Phytotoxic effects of sulfometuron-methyl have also already been observed in other tree species. Geyer and Long (1998) note that this compound negatively affects the species *Pinus strobus*, *P. sylvestris*, *P. nigra*, and *Juniperus virginiana*. Correia and Villela (2015) observed that as the concentration of this ripener was increased, there was also reduction in the height of *E. urograndis,* as well as phytointoxication when exposed to 15 g ha^{-1}concentrations of sulfometuron-methyl, while other species (cotton, peanut, soy, and bean) showed injuries that negatively reflected upon their development, as can be observed in the study from Correia and Leite (2012). Pires et al (2013) found positive effects in *E. urograndis* after applying low concentrations of this compound, with 14% gains in leaf area and 7% gains in total dry mass compared to the plants without application. Thus, varied responses found between the species are probably due to the different plant abilities to metabolize, translocate, and even compartmentalize sulfometuron-methyl (Correia and Leite, 2012).

Materials and Methods

Plant materials and growth conditions

The experiment was conducted in open and semi-controlled area, with all pots irrigated daily until reaching field capacity. During the experimental period, average air temperature of 23.1 ºC (maximum of 30.1 ºC and minimum of 17.3 ºC) was recorded, with 65.8% relative air humidity and 252.8 hours of sunlight per month.

C. myrianthum seedlings with 18 months age had average of 10 leaves, stem diameter of 5.0 mm and 20 cm in height. As experimental units, we used pots (5 L) previously filled with a mixture of soil collected in the surface layer of a Dark Red Latosol - after determining fertility -, sand, and manure (3:1:1 v:v:v). After planting, plants were acclimated for 15 days before applying the ripeners.

Treatments and experimental design

Treatments consisted of a 6x2 factorial arrangement, corresponding to six concentrations and two chemical ripeners: glyphosate (0.0; 9.6; 28.8; 57.6; 86.4; and 115.2 g e.a. ha^{-1}) and sulfometuron-methyl (0.0; 0.75; 2.25; 4.50; 6.75; and 9.00 g e.a. ha^{-1}). These doses are equivalent to 0, 5, 15, 30, 45, and 60% of commercial concentration of Glizmax® (192 g e.a. ha^{-1}) and Curavial® (15 g e.a. ha^{-1}) recommended for sugarcane.

A randomized block design with six replications was used. The subdoses of ripeners (drift simulation) were applied 15 days after planting of seedlings byusing a CO_2 pressurized costal sprayer, equipped with XR 110.02 regulated for a volume of 200 L ha^{-1} at 2.2 bar tank pressure. At the time of application, temperature was 27 ºC, with 60% cloud cover, 63.5% air humidity, and wind speed of 3 km h^{-1}.

Chl quantification and fluorescence

At 07, 30, and 60 days after the application (DAA) of ripeners, the total relative chlorophyll content (*a* + *b*) was measured using a portable chlorophyll meter (CFL 1030, Falker) and the potential quantum efficiency of the ***a*** chlorophyll (F_v/F_m) was measured using a fluorometer (PEA-MK2 Hansatech). These measurements were carried out in the second pair of totally expanded leaves (n=5).

Gas-exchange parameters

Also at 07, 30, and 60 DAA, in the second pair of fully expanded leaves, the net CO_2 assimilation rate (*A* - µmol CO_2 m^{-2} s^{-2}) was measured, as well as stomatal conductance (g_s -

mol H_2O m^{-2} s^{-2}), internal CO_2 concentration (C_i - µmol CO_2 mol^{-1}), and transpiration rate (E - mmol H_2O m^{-2} s^{-2}), using a portable Infrared Gas Analyser (IRGA - LI 6400, LiCor, Lincoln, NE, USA). For this, the photosynthetically active photon flux (quantum) was kept at 1500 µmol m^{-2} s^{-1}, the relative air humidity and temperature in the sampling chamber at 85% and 25 ºC, respectively, and the reference CO_2 level at 400 µmol mol^{-1}.

Growth parameters

At 7 and 60 DAA, morpho-physiological alterations in the plants resulting from experimental treatments were recorded photographically. At the end of the experimental period (60 DAA), the number of leaves, plants height (ruler graduated in millimeters), and stem diameter (digital pachymeter) were measured. Subsequently, leaves and stems of plants were separated and the leaf area was measured using a leaf area measurer (Li-Cor Inc., LI3000A, USA). The stems and leaves were placed in a forced air circulation oven at 70ºC for 96 hours and the dry mass subsequently determined using analytical precision scale (Marte, AS2000C, Brazil).

Statistical analysis

Statistical analyses were performed using analysis of variance (ANOVA) and means were compared using the Tukey test at a 5% probability level. When the interaction was significant, we chose to consider the effect of concentrations within each ripener separately.

Conclusion

Both glyphosate and sulfometuron-methyl caused visual symptoms of phytointoxication in *Citharexylum myrianthum*¸ which were accompanied by alterations in gas exchange up until 30 days after application. At 60 days after the application, all plants treated with glyphosate presented recovery of photosynthetic capacity, with no detrimental effect on their initial development. Although plants had recovered their photosynthetic capacity at 60 days after the application of sulfometuron-methyl, there was reduction in the height of plants treated with the highest dose, combined to super sprout resulting from the death of the apical meristem. Thus, despite there not being any significant difference in total dry mass, the development of these plants was affected.

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