RESEARCH ARTICLE

Journal of Research in Weed Science 1 (2) (2018) 90-98

Journal of Research in Weed Science

Journal Homepage: www.jrweedsci.com

Effect of electron transport rate on sugarcane submitted to pre-emergence application of atrazine and tebuthiuron

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Accepted: 25 October 2018

Abstract

The objective of this work was to assess the photosynthetic efficiency of sugarcane crop, in pre-emergency application, of two herbicides inhibiting photosynthesis. For sugar cane cuttings planting (variety SP80-3280), pots with a capacity of 8 dm^3 were used. Atrazine and tebuthiuron application was performed by using a stationary spray installed in laboratory conditions and after treatment application, plants were transported to a green house where were kept until the end of the trial. The reading of transport rate (ETR) was conducted in the middle portion of the youngest leaves of sugarcane with a portable fluorometer, with intervals for ETR assessment constituted by: 16, 17, 19, 25 and 30 days after herbicide application. It was also realized visual analysis of phytotoxiciy performed 30 days after application. The results showed that tebuthiuron herbicide caused greater reduction in the rate of electron transport than atrazine. In relation to herbicide symptoms, there was no significant difference between the studied herbicides. At the end of the work it could be verified that the chosen method using fluorometer to measure the rate of electron transport after herbicide application was adequate, allowing verifying sugar cane crop intoxication, even before any visual detection of herbicides performance in plants.

Keywords: ETR, fluorometer, herbicide, intoxication, selectivity

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1. Introduction

The presence of weeds could reduce the yield and quality of sugarcane in agricultural systems. This reduction occurs mainly through competition for nutrients, light and water lodging of pests and diseases, and the release of allelochemicals. In addition, the presence of weeds makes it difficult to cut sugarcane stalks at harvest time, causing the industrial yield to decrease resulting in the loss of sugarcane quality (Negrisoli et al., 2004). According to Freitas et al. (2004), in the current condition of sugarcane production, the chemical method is the most practical way to weeds control in sugarcane due to the extensive cultivated areas, shortage of labor, ease of application, cost and effectiveness of the treatment, in addition to being a cost effective and high-performance method, compared to others. The proper application of herbicides is fundamental so that it reaches its target in order to carry out the control without causing phytotoxication problems in the crop or environmental contamination. However, due to the anatomical and physiological similarities between weeds and cultivated species, the risk of phytotoxicity of agrochemicals in agricultural crops always occurs when the control is adopted with herbicides.

The ability of a given herbicide to eliminate weeds in a crop, without reducing its productivity and the quality of the final product obtained, is called selectivity (Negrisoli et al., 2004). Selectivity can not be determined solely by the simple verification of visual symptoms of intoxication since examples of herbicides are known which can reduce the productivity of crops without, however, producing them visually detectable effects and also examples of herbicides which cause quite severe injuries, but they allow them to fully express their productive potentials (Negrisoli et al., 2004). To control the main weeds and avoid possible damage to the sugarcane crop, many herbicides with different active ingredients and formulations are registered for use in Brazil. Among them, atrazine and tebuthiuron are widely used herbicides (Rodrigues & Almeida, 2005). The herbicides tebuthiuron and atrazine because they have as mechanism of action the inhibition of photosystem II, bind to protein D1, which is the binding site of QB, and located in the membranes of thylakoids of chloroplasts, causing, consequently, blockage of the electron transport from QA to QB. This reaction reduces CO_2 fixation, the production of ATP and $NADPH_2$, which are essential for plant growth (Breitenbach et al., 2001).

In relation to the electron transport phase during photosynthesis in plants, light is absorbed by pigments of the antenna complex, which excite the electrons, transfer energy to the reaction centers of photosystems II and I (Young and Frank, 1996). When excess energy occurs, it can be dissipated as fluorescence (Krause and Winter, 1996). One of the ways of monitoring the inhibition or reduction of electron transfer between herbicide photosynthesis systems, which can be observed even in intact leaves, is the chlorophyll fluorescence (Maxwell and Johnson, 2000); being that the reduction in energy dissipation by the photochemical process is reflected by corresponding increase in fluorescence. Fluorescence analysis has been widely used in the understanding of photosynthetic mechanisms, mainly helping to measure these mechanisms after application of herbicides (Ireland et al., 1986). The tool available for such measurement is the fluorometer, capable of quickly identifying the injuries caused to the photosynthetic apparatus, even though the symptoms of intoxication are not visually detected.

The present work had the objective of evaluating the photosynthetic efficiency in sugarcane with the application of pre-emergence photosystem II inhibitor herbicides.

2. Materials and Methods

A greenhouse experiment was conducted at the Center for Advanced Mathematical Research (NUPAM), belonging to the Faculty of Agronomic Sciences of Botucatu / SP, at the following geographical coordinates: latitude 22 ° 07'56 " S, longitude 74 ° 66'84 " W Gr. and altitude of 762 m.

The soil used as substrate was initially dried in the shade for a period of 48 hours and then sieved in a 200 mesh sieve and subjected to chemical and physical analysis (Table 1). The fertilization was carried out based on the results of the soil analysis, then the sugar cane tots were planted in pots of 8 L capacity (variety SP80 -3280).

Table 1 - Chemical and physical analysis of the soil used in the experiment.

Soil	pH ($CaCl_2$)	O.M ($g\ dm^{-3}$)	P res. ($mg\ dm^{-3}$)	K^+	Ca^{+2}	Mg^{+2}	H^++Al^{+3}	SB	T	V(%)
				($mmol_c\ dm^{-3}$)						
LVd	4,3	19,0	1,0	0,6	10,0	4,0	5,8	14,6	73	21

Granulometry (%)	Soil Class						
	Clay	Silte	Sand				Textural class
			Thin	Medium	Thick	Total	
LVd	20,0	4,0	22,9	35,7	17,4	76,0	Medium

The experiment was installed in a completely randomized design with four replicates in a factorial scheme, the first herbicide factor being applied in pre-emergence (tebuthiuron 1.0 kg ha^{-1} and atrazine 2.50 kg ha^{-1}) and the second reading interval of the electron transport rate (ETR), described later, in addition to a control without herbicide. The application of the herbicides was performed by means of a stationary sprayer installed in the laboratory and equipped with a bar containing four ends of type XR110.02. The spraying was carried out on a constant pressure of 1.5 bar, pressurized by compressed air, with a consumption of 200 L ha^{-1}. The temperature at the time of application was 25 ° C with relative humidity of 70%. After the treatments were applied, the plants were transported to greenhouse where they remained until the end of the test, being carried out the evaluations of the rate of electron transport and visual analysis of phytointoxication. The visual evaluation of intoxication of sugarcane plants was performed 30 days after the application of the herbicides through a scale of percentage notes, where "0" corresponded to no injury and "100" the death of plants (SBCPD, 1995).

The electron transfer rate (ETR) in the medium portion of the sugarcane leaves was measured using the Multi-Mode Chlorophyll Fluorometer Model OS5p (Opti-Sciences) in the intervals of 16, 17, 19, 25, and 30 days after the emergence. The fluorescence in the apparatus was to the diode source with peak of red light in the wavelength of 660 nm being blocked radiations greater than 690 nm. The mean light intensity was adjusted to the range of 0 to 1 $\mu Molm^{-2}s^{-1}$ using the 35 W halogen lamp. The light beam was optically monitored inside the chamber to correct variations due to changes in the appliance. The optical signals were transferred to the surface of the sheet by a custom fiber optic trifurcation, with the area illuminated using Yield protocol of 2 cm^2. The re-emitted light was conducted via optical fiber to the apparatus through three connectors that connect to the side of the OS5p. The analyses were done following the methodology of Genty et al. (1989), evaluating the emission of chlorophyll fluorescence on the adaxial side of the leaves. The Yield protocol records the effective quantum measurements produced in photosystem II (PSII). Both the source of sunlight and artificial light can be used to direct photosynthesis.

Electron transport rate (ETR) - μMols electrons.$m^{-2}.s^{-1}$ = (Y) (PAR) (0.84) (0.5) is equivalent to: (quantum production of PSII) x (measures of photosynthetically active radiation measured in μMols electrons.$m^{-2}.s^{-1}$) x (leaf absorption coefficient) x (fraction of light absorbed by the PSII antenna complex). The ETR is a measure of the separation of charges from the PSII reaction center. In the equation standard values are used, however, both the absorption and fraction of light absorbed by the PSII can be changed. Although 0.84 is an average value for many plant species, studies have shown that the leaf absorption coefficient can vary with light quality, species, chlorophyll content and leaf reflectance. For the light fraction that is absorbed by the antenna complex of the PSII the works show that variation occurs according to the species and extends from 0.42 to 0.60 (Laisk and Loreto, 1996). As the ETR parameter determines the rate of electron transport in the PSII, the use of the ETR value allows detecting the effect of the herbicide performance at concentration level of 0.5 micromoles dm^{-3}, while the traditional method, which includes (Figure 2), which can be used to measure the Fv / Fm parameter, which can be detected only at a concentration level that is 100 times higher (Korres et al., 2003; Abbaspor et al., 2006).

The photosynthetic efficiency data were expressed as a percentage of the control and were submitted to analysis of variance. The means compared by the t-test at 10% significance (Velini et al., 2006).

3. Results and Discussion

The synthesis of the analysis of variance and test of means for the data of ETR, analysed as function of the herbicides and the time, are exposed in Table 2. The isolated effects of time (days) were significant at 1% probability and only at 25 days after application (DAA) of the herbicides, the greatest reduction of the ETR was presented in relation to the other evaluated intervals. However, the effect of each herbicide, with mean time in days, was also significant at 1%, with the herbicide tebuthiuron showing a greater reduction of the ETR. The interaction of the two factors, time and herbicides, was also significant at 1%.

Table 2- Synthesis of variance analysis and mean test for ETR.

Time (Days after application)	ETR
16	94,32 b
17	94,83 c
19	92,39 b
25	88,75 a
30	95,24 c
Herbicide (H)	
No control	100,00 c
Atrazine	96,89 b
Tebuthiuron	82,44 a
Teste F	
T	10,70**
H	219,94**
T x H	4,38**
Coefficient of variation (%)	4,29

* In each column, for each factor, averages followed by the same lowercase letter do not differ, each other, by the t test, at 10% probability. NS: Not significant (P <0.05); *: Significant (P <0.05); **: Significant (P <0.01).

From the interaction of all herbicides with the period in days (Table 3) it was observed, at 16 DAA, that tebuthiuron presented with greater reduction of the ETR compared to atrazine. At 17 DAA, tebuthiuron maintained the inhibition of electron transport relative to atrazine. The same occurred at 19 and 25 DAA, however, at 30 DAA, recovery of the ETR occurred in the plants that developed in soil submitted to the application of tebuthiuron.

By monitoring the electron transport in the sugarcane plants, a higher initial effect of reducing the ETR was observed after application of tebuthiuron. This is due to the fact that the active ingredient of tebuthiuron acts in inhibiting the transport of electrons in the PSII, precisely where the fluorescence emitted by the photosynthetic apparatus after detection is detected through the fluorometer readings. Atrazine also has the same mechanism of action, inhibitor of PSII, however, the plants submitted to such product did not present this initial intoxication as plants treated with tebuthiuron. In a study developed by Girotto et al. (2010), with post emergence application of atrazine, it was also detected low intoxication, through the ETR, for the cultivar SP80 3280, when compared to the herbicide S-metolachlor. Souza et al. (2009) evaluate the tolerance of sugarcane cultivars to herbicides applied in post-emergence in the crop sock. It was detected a small reduction in PSII electron transport after the application of diuron + hexazinone in the initial phase of crop development, but not enough to impair the height, stand, production and technological quality of the cultivars studied.

In a study developed by Dayan et al. (2009), the ETR was monitored in maize plants when submitted to the application of amicarbazone and atrazine. The electron transport rate maintained a reduction of approximately 70% and 30% of the photosynthetic ETR with 24 hours after the application respectively of amicarbazone and atrazine, the corn being more tolerant to atrazine when compared to amicarbazone, under the conditions studied. Thus, the use of the fluorometer becomes an interesting tool for the early identification of physiological injuries (from 25 to 30 days after application to the herbicides under study at work), a fact not identified through visual analysis of phytotoxification. Some oscillations in the ETR values for the tebuthiuron treatment should possibly have some effect of the environment. The small oscillations of the ETR occurring during the evaluated period are due to environmental conditions, since the photosynthetic capacity of the plants can be altered by biotic or abiotic stresses, such as changes in temperature, radiation, water deficiency, etc. (Bown et al. 2002).

Table 3- Electron transport rate (ETR) responses, as a function of time interaction after application and herbicides used.

Herbicides	**Time (Days after application)**				
	16	**17**	**19**	**25**	**30**
No control	100,00Ab	100,00Ab	100,00Ac	100,00Ac	100,00Ab
Atrazine	98,23Bb	97,56Bb	95,78ABb	93,42Ab	99,45Ba
Tebuthiuron	84,73BCa	86,92Ca	81,40Ba	72,85Aa	86,30Ca

** Means followed by distinct upper case letters differ statistically from one another by the t test (p> 0.10), on the line. * Means followed by distinct lowercase letters differ statistically from each other by the t test (p> 0.10) in the column.

In relation to the reduction of electron transport, the application of tebuthiuron presented a greater reduction when compared to the herbicide atrazine. However, this reduction did not affect the initial development of sugarcane, which was verified by the analysis of phytointoxication. The mean data for each treatment with herbicide and phytotoxicity symptoms (Figure 1) were observed in plants treated with atrazine with approximately 10% of phytotoxification, and 8% for tebuthiuron, and did not differ significantly from each other.

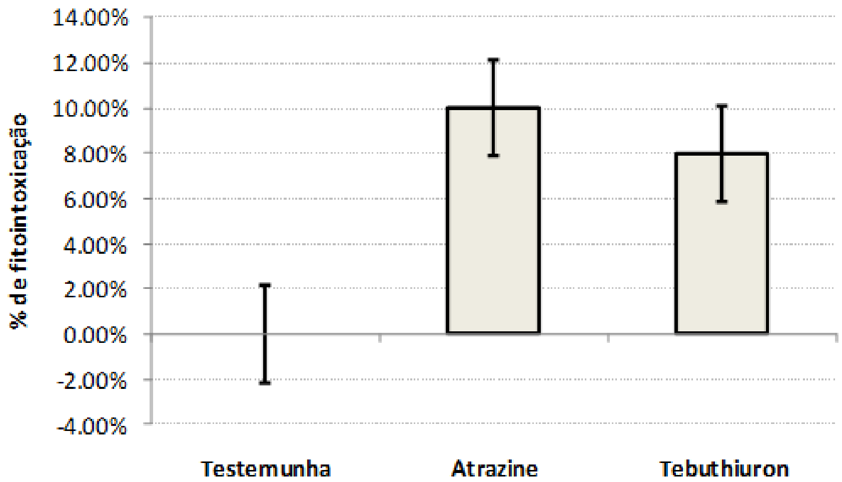

Figure 1- Percentage of phytointoxication at the 30 DAA of the products.

The sugarcane plants showed different sensitivity to treatments, especially tebuthiuron, which showed a greater reduction of the ETR in the conditions and periods studied. In general, the methodology used with the fluorometer was adequate to verify the action of photosystem II inhibitory herbicides by means of the instantaneous verification of electron transport, the initial phase of photosynthesis in plants. And, with the application of tebuthiuron in cultivar SP80 3280 of sugarcane, it was verified the intoxication in the culture even before any visual detection of the herbicide performance in the plants.

Conclusion

The sugarcane cultivar SP80-3280 presented a good selectivity to the herbicides tebuthiuron and atrazine applied in pre-emergence. The greatest reductions of the ETR were observed in the plants that developed in soil that received the application of tebuthiuron, and the fluorometer was an alternative to detect the insults of herbicides early in the sugarcane crop.

Conflict of interest

Authors declare no conflicts of interest for this study.

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Cite this article as:
Girotto Marcelo. 2018. Effect of electron transport rate on sugarcane submitted to pre-emergence application of atrazine and tebuthiuron. Journal of Research in Weed Science. 1(2): 90-98.