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Physiological aspects of corn plants related to mesotrione herbicide selectivity

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Abstract

The objective of the present study was to assess the physiological aspects related to the activity of mesotrione herbicide at different doses in corn plants. The experiment was performed, in a greenhouse, on UENF 506-8 hybrid grown in 12 L pots with substrate (2:1 sand and clay). A randomized complete block design in a 3 x 5 factorial scheme, with four replications was used. Plants at fourth leaf stage were exposed to three doses (0, 0.15 and 0.30 kg ha-1) of herbicide mesotrione. Three, four, five, six and 13 days after application (DAA) of herbicide mesotrione gas exchange and chlorophyll *a* fluorescence (considering a JIP_{Test} analysis) were measured. Plant height (PH), leaf area (LA), canopy (CDM) and root dry matter were measured at 28 DAA, when plants were at V12 stage and *ca*. 85 to 90% grown of its total area. The results showed that UENF 506-8corn hybrid was remarkably tolerant to dose of 0.15 Kg ha^{-1} without deleterious effects on photochemistry and biochemistry path way, maintaining the values of several JIP_{Test} parameters and gas exchange. However, decreases in photosynthetic rate, stomata conductance, and transpiration and several JIP_{Test} parameters were observed when the plants were exposed to dose of 0.30 kg ha^{-1} although there was no harmful damage on most of the growth parameters. However, dose of 0.15 Kg ha^{-1} should be recommended since dose as high as 0.30 kg ha^{-1} caused decrease in CDM.

Keywords: Herbicide; *Zea mays* L.; Phytotoxicity.

Abbreviation: Qa_quinone a, ET_electron transport, DAA_day after application. *A*_ photosynthesis rate, *gs*_stomata conductance**,** *E*_transpiration rate, C_i/C_a_internal-to-ambient CO_2 concentration ratio, F_v/F_m _ maximum PSII efficiency, ABS/RC_amount of energy absorbed by the pigments in the PSII antenna complex, per unit of active reaction center, DI_o/RC_dissipated energy per unit of active reaction center, RC/CS_o_number of active reaction centres per cross, ET_o/CS_o_ energy distribution ratio per transversal section from sample, PH_plant height, LA_leaf area, CDM_canopy dry matter, RDM_root dry matter.

Introduction

Brazil is one of the main corn (*Zea mays* L.) producers with an extensive cultivated area and volume produced of *ca*.85 million tons in 2014/2015 harvest, with mean yield of 5,716 kg.ha^{-1} (CONAB, 2016).

Weeds can cause losses in corn yield ranging from 13.1 to more than 85% depending on the competing species, degree of infestation, times and extension of weed coexistence period, the development crop stage and the climatic conditions (Freitas et al., 2009). Therefore, weed control is an economic necessity and the chemical control has been the most common method used on large areas. However, the efficiency varies among herbicides depending on the environmental conditions, time of application and weed species infesting the crop (Freitas et al., 2014).

Gas exchange measurement is a powerful tool for studying herbicide selectivity/phytotoxicity in corn plants since it demonstrates photosynthetic performance (Lemos et al., 2012). Chlorophyll *a* fluorescence have been widely used because is a non-destructive method and also allows qualitative and quantitative analysis of the absorption, partitioning and use of the light energy in photosystem II (PSII) and possible effects on photosynthetic capacity (Mouget and Tremblin, 2002; Netto et al., 2005). This technique has been used to investigate the photochemical and non-photochemical processes which occur during the light absorption and the factors related to the absorption capacity and transference of the light energy through electron transport chain (Genty et al., 1989; Krause and Weis, 1991).().,Changes in the fluorescence signal result from variations in the photochemical and non-photochemical quenching showing either absence or presence of damage on the photosynthesis process (Mouget and Tremblin., 2002).

The JIP_{Test} variables are derived from chlorophyll *a* fluorescence and have been used for *in vivo* investigations of the performance of the photosynthetic apparatus, since it is sensitive to stress caused by changes in environmental conditions (Martinazzo et al., 2012; Silvestre et al., 2014; Bussotti et al., 2014).The JIP_{Test} shows how the photon energy is: 1) absorbed by pigments in photosynthetic antenna system (ABS); 2)dissipated in the antenna system in the form of heat (DI);, 3)released in the form of fluorescence (under longer wavelength), 4) channeled to the reaction center to reduce quinone a (Qa) e.g., Qa to Qa^- (TR). $Qa^-$is re-oxidized to create the electron transport (ET), what provides reducing power for carbon assimilation by the Calvin cycle. The JIP_{Test} parameters have been shown to be adequate for environmental stress studies (Silvestres et al., 2014) and further, can help in plant genetic breeding for different environmental conditions, including corn breeding (Simic et al., 2014).

Mesotrione is a selective and systemic herbicide applied post emergence that may indicated to control weeds infesting corn crop, (Sprague et al., 1999; Rodrigues and Almeida, 2005). Plants absorb the herbicide mainly through the leaf and root organs via apoplastic movement (Mitchell et al., 2001). Plants which are susceptible to mesotrione present leaf chlorosis three days after application evolving to widespread necrosis and death of the plant within 2 weeks (Pallet et al., 1998; Wichert et al., 1999). Overall, corn plants are tolerant to mesotrione herbicide (Johnson et al., 2002), mainly related to the recommended dose. However, high ones can overcome the tolerant mechanisms and cause physiological damage linked to decreases in crop yield. Therefore, we aimed at investigating physiological and growth characteristics in corn plants exposed to different doses of mesotrione herbicide.

Results

Leaf gas exchange performance

A values averaged *ca.* 35 µmol CO_2 m^{-2} s^{-1} at three DAA and was similar at doses of 0 and 0.15 kg $ha^{-1.}$ (Fig 2A) However, there was decrease in carbon assimilation at dose of 0.30 kg ha^{-1} with photosynthetic rates below 10 µmol CO_2 m^{-2} s^{-1}, but similar to the control at 13 DAA. Regarding the time, the highest photosynthetic rates occurred at six DAA at doses of 0 and 0.15 kg ha^{-1} while at dose of 0.30 kg ha^{-1} occurred at 13 DAA (Fig 2A).

Gs values was similar among doses at three DAA with values around 0.13 mmol H_2O m^{-2} s^{-1}, however, plants exposed to dose of 0.30 kg ha^{-1} showed lower stomatal aperture values than 0 and 0.15 kg ha^{-1} doses from the three up to six DAA (Fig 2B). *Gs* values were similar among doses at 13 DAA with values around 0.27 mmol H_2O m^{-2} s^{-1}. After three DAA, stomata conductance showed increased values for at all doses, with similar values between 0 and 0.15 kg ha^{-1}doses (Fig2B).

E values were similar among doses at three DAA (*ca.* 3 mmol H_2O m^{-2} s^{-1}) however, plants exposed to doses of 0.30kg ha^{-1} showed lower values from the four up to six DAA, but similar values to the control at 13 DAA (Fig 3C).

C*i*/*Ca* ratio values were similar among doses at three DAA albeit it were greater at four and five DAA (*ca.* 0.36 and0.38, respectively) at doses of 0.30kg ha^{-1}. *Ci/ca* ration values were similar among doses at six DAA (Fig 2D).

Chlorophyll a fluorescence analysis through the JIP_{Test} and growth

ABC/RC values were similar among doses except at four DAA when the plants exposed to dose of 0.30 kg ha^{-1} showed higher values (5) than 0 and 0.15 kg ha^{-1}doses (3.5).. Regarding the evaluation period, the highest values were observed at three DAA in plants exposed to dose of 0 and 0.15 kg ha^{-1}, while the plants exposed to dose of 0.30 kg ha^{-1} showed the highest values at four DAA.(Fig 3A).

DIo/Rc values were similar at three DAA among doses, except at four DAA when the plants exposed to dose of 0.30 kg ha^{-1} showed higher values (*ca.* 2.5) albeit at five DAA similar values were among dose, with values around 1.3 at 13 DAA. Plants exposed observed to dose of 0 and 0.15 kg ha^{-1} showed highest values at three DAA, while the plants exposed to dose of 0.30 kg ha^{-1}showed the highest values at four DAA (Fig 3B).

Similar RC/CSo values were observed at three DAA among doses, however, the plants exposed to dose of 0.30 kg ha^{-1}showed lower values at four and five DAA (*ca.* 98 and 118, respectively). After six DAA similar results o among doses were observed, with values around 120 at 13 DAA. Plants exposed to dose of 0 and 0.15 kg ha^{-1}showed the highest values at five and six DAA, while the plants exposed to dose of 0.30 kg ha^{-1} the highest values occurred at six and 13 DAA (Fig3C).

ET/CSo values among doses were observed throughout the assessment period and the highest values occurred after the five DAA, with values reaching *ca.* 140 at 13 DAA (Fig 3D). Similar values among doses were observed for the F_v/F_m ratio (*ca.* 0.76), except t four DAA when the plants exposed to dose of 0.30 kg ha^- showed lower value (ca. 0.5). (Fig 3E). There were no significant differences for NF, PH, RDM and LA traits. However, plants exposed to dose of 0 and 0.15 kg ha^{-1} showed the highest values for CDM trait (Table 1).

Discussion

Corn plants were exposed to doses of mesotrione herbicide (0, 0.15 and 0.30 Kg ha^{-1}) to study the effects of ones on physiological aspect and growth traits. Increase in *A* values probably occurred by increasing the amount of available light (Fig 1). In fact, increase in photosynthesis rates in corn plants linked to the increase in photosynthetically active radiation were observed, ranging with the phenological stage Lopes et al. (2009). Photosynthesis rates obtained in the present study are similar to those found elsewhere, taking into account different varieties and phenological stages (Lopes et. al., 2009; Lemos et al., 2012). Corn is a mesotrione -tolerant crop due to its metabolic capacity to detoxify these molecules, producing compounds without herbicide activity (Johnson et al., 2002). Indeed plants exposed to dose of 0.15 kg ha^{-1} showed no deleterious effects on gas exchange and growth parameters. However, plants exposed to dose of 0.30 kg ha^{-1}showed remarkably decreases in gas exchange-related traits up to six DAA, indicating that metabolic capacity to mesotrione detoxification is dose-dependent.

Gs and *E* changes were accompanied by a similar variation pattern in *A*, indicating some stomatal limitation at 0.30 kg ha^{-1} dose. Crop yield and stomata functioning are intimately related, since the CO_2 uptake is controlled by stomatal aperture (Costa and Marenco,2007).

Transpiration depends mainly on stomata conductance, which decreases according to the incident photosynthetic active radiation and the amount of water available (Bergonci and Pereira, 2002). However, the C_i/C_a ratio showed similar values at 0.30 kg ha^{-1} dose throughout the assessment period, indicating that other limitations (non-stomatal), in addition to those stomatal, contributed to decreases the *A* values. In fact, increase in photosynthesis rate causes fall in the internal CO_2 concentration exercising a strong retro-active effect and can consequently cause fall in the photosynthesis rate (Machado and Lagoa, 1994). ,

Leaf-air vapor pressure gradients are the driving force for the vapor flow consequently affecting the stomata and transpiration system (Taiz and Zaiger, 2013). Thus, some stomata limitation may have occurred for all the doses since the vapour pressure deficit were somewhat high (data not

Table 1. Analysis of variance for number of leaves (NF), plant height (PH), canopy (CDM) and root dry matter (RDM), and leaf area (LA) from UENF 506-8 cultivar corn plants exposed to doses of 0.15 and 0.30 kg ha^{-1} of mesotrione herbicide at 28 days after application.

Treatments	NF^{ns}	PH^{ns}	RDM^{ns}	$MSPA^{**}$	LA^{ns}
0 (Control)	8.75±0.14	0.92±0.016	8.54±0.58	14.5±0.14	1826.5±93.75
0.15 kg ha^{-1}	8.25±0.12	0.88±0.091	13.17±0.65	13.75±0.37	1839.8±61.50
0.30 kg ha^{-1}	8.25±0.37	0.86±0.007	9.19±0.03	10.75±0.13	1905.6±59.61
CV (%)	12.16	5.98	31.89	6.70	15.39

Each value represents the mean ± SE (n=4). **significant and ^{ns}not significant by the F test (p≤0.01). Each value represents a mean ± SD (n=4). CV=coefficient of variation.

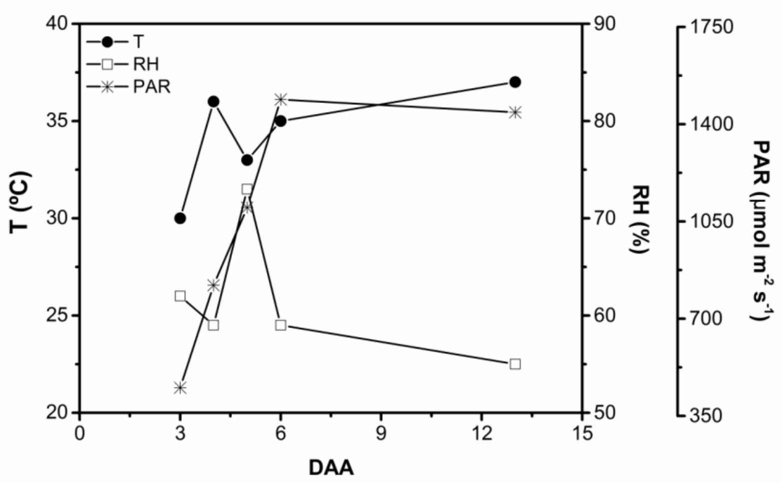

Fig 1. Average temperature (T), relative humidity (RH) and maximum photosynthetic active radiation (PAR) during the experimental period.

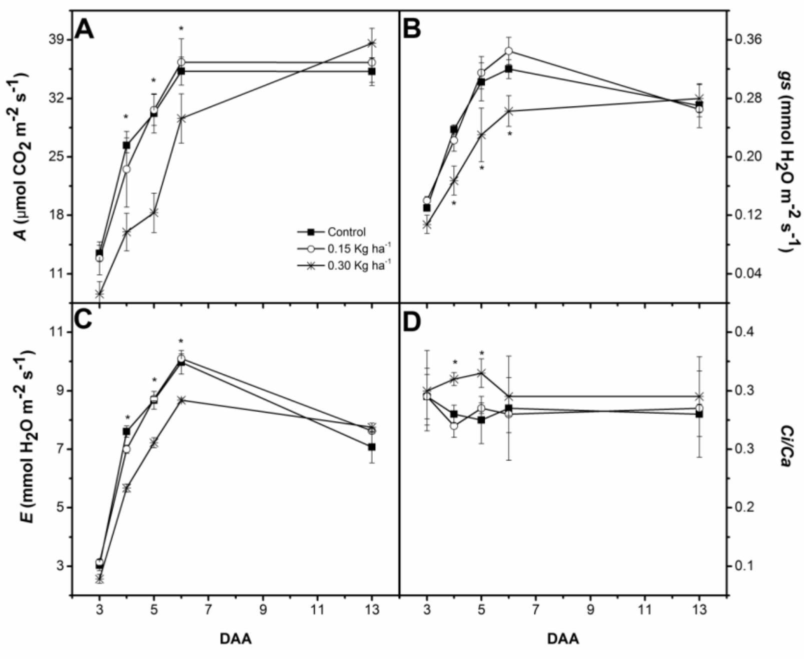

Fig 2. Variation in photosynthesis rate-*A*- (A), stomata conductance-g_s (B), internal-to-ambient CO_2 concentration ratio-C_i/C_a, (C) and transpiration rate-*E* (D) obtained between 8 AM and 9 AM on UENF 506-8 cultivar plants exposed to doses of 0.15 and 0.30 kg ha^{-1} of mesotrione herbicide at three, four, five, six and 13 days after application (DAA). Each value represents the mean ± SE (n=4). *Significant difference.

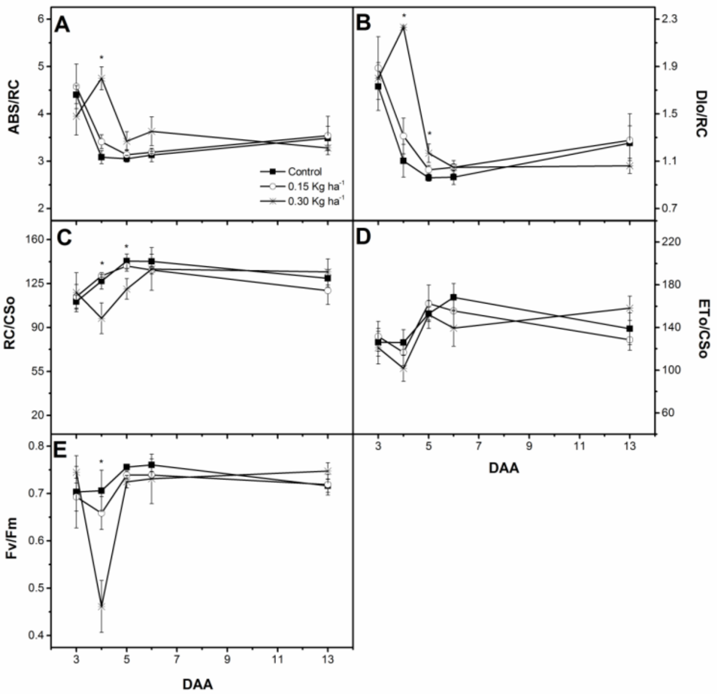

Fig 3. Variation in amount of energy absorbed by the pigments in the PSII antenna complex, per unit of active reaction center-ABS/RC (A), dissipated energy per unit of active reaction center-DI_o/RC (B), number of active reaction centres per cross-RC/CSo (C) and energy distribution ratio per transversal section from sample -ET_o/CS_o (D) and maximum PSII efficiency-F_v/F_m (E), obtained between 8 AM and 9 AM on UENF 506-8 cultivar plants exposed to doses of 0.15 and 0.30 kg ha^{-1} of mesotrione herbicide at three, four, five, six and 13 days after application (DAA). Each value represents the mean ± SE (n=4). * Significant difference.

showed) , probably due to the high temperatures reported throughout the experimental period (Fig 1). Gas exchange data indicate greater non-stomata limitations on photosynthesis. In fact, several JIP_{Test} parameters provide useful information, showing changes in light absorption and partitioning. Plants exposed to dose of 0.30 kg ha^{-1} showed a slight trend to decreased values of ET_0/CS_0 linked to the increases in ABS/RC and RC/CSo values. These changes can result in DI_0/RC increase as observed in *Lathyrus* genus and act as a protection mechanism to maintain the F_v/F_m ratio (Silvestre et al., 2014), which has shown to be sensitive variable and useful for studies of mesotrione in popcorn (Freitas et al., 2014).. However, such changes were not sufficient to maintain F_v/F_m ratio in corn plants, showing that the reduction in photosynthesis rates was linked mainly to photochemical limitations. Plants exposed to dose of 0.30 kg ha^{-1} showed lower CDM values although it had recovered F_v/F_m ratio values at 13 DAA (Table 1). On the other hand, plants exposed to dose of 0.24 kg ha^{-1} have not been altered both canopy and root dry matter (Procópio et al. (2006)). Furthermore, Fritsche-Neto et al. (2010) did not observe positive correlation between CDM and yield. Our data indicate that the recommended mesotrione dose (0.12 kg ha^{-1}) did not affect the gas exchange and chlorophyll *a* fluorescence parameters in the UENF 506-8 cultivar, as observed for others corn cultivars (Johnson et al., 2002; Procópio et al., 2006). However, doses as high as 0.30kg ha^{-1}, which might be used due to negligence while preparing the solution or lack of information and instructions, affected the photosynthesis pathway. Although the UENF 506-8 cultivar plants recovered its gas exchange and chlorophyll *a* fluorescence values,, the period in which the plants presented the lowest *A* values can affect yield as observed in popcorn plants (Freitas et al., 2014).

Materials and Methods

Plant materials conditions and experimental design

The experiment was carried out in a greenhouse using UENF 506-8 hybrid corn plants grown in 12 L pots containing substrate (2:1 sand and clay) and fertilized with 40 g pot^{-1} of N-P-K (4:14:8), ratio with one plant per pot. The plants were irrigated daily and the moisture content was kept close to field capacity. A randomized complete block design in a 3 x 5 factorial scheme, with four replications was used.

Plants at fourth leaf stage were exposed to three doses (0, 0.15 and 0.30 kg ha^{-}1) of herbicide mesotrione, using a backpack sprayer, pressurized with CO_2, with constant 3.0 kgf cm^{-2} pressure and equipped with a Teejet DG 80.02 nozzle, calibrated to apply the equivalent of 237 L of solution per hectare (ha). The applications were made on days with full sunlight , relative humidity of *ca.*75% and wind speed close to 4 km h^{-1}. After three, four, five, six and 13 days after application (DAA) of mesotrione herbicide, gas exchange, chlorophyll *a* fluorescence (considering a JIP_{Test} analysis) were measured

Gas exchange and fluorescence measurements

Gas exchange (photosynthesis rate-*A*, stomata conductance-*gs*, transpiration rate –*E*, as well as the internal-to-ambient CO_2 concentration ratio-C_i/C_a) were determined between 8.00 AM and 9:00 AM, using a portable open-system infrared gas analyser (LI-6200, Li-COR, Lincoln, Nebraska, USA), with leaf measuring area of *ca.* 6.8 cm^2. The average environmental conditions during the assessment days are shown in Fig1.

Chlorophyll *a* fluorescence was evaluated at 8 AM on the same non-detached leaves used for the gas exchange measurements, using a Pocket PEA fluorometer (Hansatech, King's Lynn, UK), taking the measurements on the two last leaves (from the tip to the base of the plant) with one measurement per leaf. The leaf was dark-adapted for *ca*. 30-40 min. using leaf clips (Hansatech) to turn the reaction centres into an "open" (oxidised Q_A) state (Bòlhar-Nordenkamphet al., 1989). Thereafter, the leaf samples were exposed to saturating irradiance (3500 000 μmols m^{-2} s^{-1}) over a 4 mm diameter area to obtain the Chl *a* PSII fast fluorescence kinetic transients (OJIP).

In addition to the F_v /$F_m$variable which represents the maximum PSII efficiency, some keys parameters of photosynthetic process (JIP_{test} analysis) were obtained using a Biolyzer computer program from chlorophyll *a* fluorescence (R.J. Strasser, Universityof Geneva, Laboratory of Bioenergetics, Switzerland), according to Strasser et al. (2000): ABS/RC (reflecting the amount of energy absorbed by the pigments in the PSII antenna complex, per unit of active reaction center), DI_o/RC (reflecting the dissipated energy per unit of active reaction center), RC/CS_o (reflecting the number of active reaction centres per cross) and ET_o/CS_o (reflecting the energy distribution ratio per transversal section from sample).

Growth parameters and statistical analyzes

After 28 days DAA when the plants were at V12 stage and with *ca.* 85 to 90% grown of its total area, plant height (PH), leaf area (LA), canopy (CDM) and root dry matter (RDM), were assessed. The biometric data were subjected to analysis of variance and the means compared by the Tukey test ($p \leq 0.05$) using the Genes software (Cruz, 2013).

Conclusions

UENF 506-8 cultivar plants were exposed to two doses of mesotrione, showing that doses as high as 0.30 Kg ha^{-1} decrease the photosynthetic rates , however the plants recovered ones at 13 DAA. Unlike, plants exposed to dose of 0.15 kg ha^{-1} did not present alterations in the gas exchange, chlorophyll a fluorescence and growth parameters. Although plants exposed to doses of 0.30 kg ha^{-1} have recovered its gas exchange rate and chlorophyll a fluorescence parameters, a decrease in CDM was observed. . Finally, dose of 0.15 Kg ha^{-1} should be recommended since under field conditions, environment stress can potentiate to a greater extent the deleterious effects from utilization of dose as high as 0.30 kg ha^{-1}.

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