



## Article

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## DOES SPRAYING OF ATRAZINE ON TRIAZINE-RESISTANT CANOLA HYBRID IMPAIR PHOTOSYNTHETIC PROCESSES?

*A Aplicação de Atrazina em Híbrido de Canola Resistente a Triazinas Prejudica os Processos Fotossintéticos?*

**ABSTRACT** - Canola is an important rotation crop for the winter season and the use of atrazine-resistant hybrids can lead to an increase in yield. This work was aimed at evaluating the effect of atrazine on photochemical and biochemical processes of photosynthesis in triazine-resistant canola. The experiment was conducted in a greenhouse, with triazine-resistant hybrid Hyola® 555TT, in a randomized block design with three replications. The treatments consisted of application or no application of atrazine on canola plants. The plants were assessed at one, three, five, and eight days after application (DAA) for chlorophyll indexes, modulated chlorophyll a fluorescence and gas exchange. Chlorophyll indexes were higher in canola plants treated with atrazine. Application of atrazine caused an increase in fluorescence at steady state and a reduction in quantum efficiency of photosystem II and electron transport rate, at 1 DAA, and a reduction in photochemical quenching, at 1 and 3 DAA. Lower stomatal conductance, at 1 DAA, and higher net carbon assimilation rate, at 8 DAA, were found in plants treated with atrazine. The application of atrazine temporarily reduces electron transport between photosystems and increases chlorophyll indexes in resistant canola plants, raising the net carbon assimilation rate at eight days after application.

**Keywords:** *Brassica napus*, chlorophyll indexes, gas exchange, modulated fluorescence.

**RESUMO** - A canola é uma importante cultura para rotação na estação fria, e o uso de híbridos com resistência à atrazina pode contribuir para o aumento da produtividade. Este trabalho objetivou avaliar o efeito da atrazina nos processos fotoquímicos e bioquímicos da fotossíntese em canola resistente a triazinas. O experimento foi conduzido em casa de vegetação, com o híbrido resistente à triazina Hyola® 555TT, no delineamento em blocos casualizados com três repetições. Os tratamentos consistiram da aplicação ou não de atrazina nas plantas de canola. As plantas foram avaliadas em um, três, cinco e oito dias após a aplicação (DAA) quanto aos índices de clorofila, fluorescência modulada da clorofila a e troca de gases. Os índices de clorofila foram maiores nas plantas de canola tratadas com atrazina. A aplicação deste herbicida ocasionou aumento na fluorescência em estado estável e redução na eficiência quântica do fotossistema II e na taxa de transporte de elétrons, em 1 DAA, bem como redução na dissipação fotoquímica, em 1 e 3 DAA. Menor condutância estomática, em 1 DAA, e maior taxa de assimilação líquida de carbono, em 8 DAA, foram observados em plantas tratadas com atrazina. A aplicação de atrazina temporariamente o transporte de elétrons entre os fotossistemas e aumenta os índices de clorofila em plantas de canola resistentes, aumentando a taxa de assimilação líquida de carbono aos oito dias após a aplicação.

**Palavras-chave:** *Brassica napus*, índices de clorofila, troca de gases, fluorescência modulada.

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## INTRODUCTION

Control of invasive weeds in canola (*Brassica napus* L. var. *oleifera*), especially of turnip, which belongs to the same family (Brassicaceae), affects development and reduces yield. Thus, use of triazine-resistant (TT) hybrids is an important alternative for weed management. In Brazil, these hybrids are still being tested and will likely be registered in forthcoming years.

Triazine-resistant canola was originated from *Brassica rapa* L. by selection in response to herbicide spraying in the field and stable gene introgression into the crop through recurrent backcrossing between similar weeds and the crop (Tranel and Horvath, 2009). There are at least eight mutations at the target site for triazine resistance, but replacement of serine with glycine at codon 264 of the *psbA* gene, which encodes for protein D1, is the most common type of mutation found in weeds (Friesen and Powles, 2007). This type of resistance takes place because of a mutation in the chloroplast genome, where the *psbA* gene is encoded; thus, it is 100% maternally inherited and can only be disseminated by seeds, rather than by pollen (Friesen and Powles, 2007; Tranel and Horvath, 2009).

Triazines bind to protein D1 (a stable secondary electron acceptor of photosystem II (PSII) found in the thylakoid membrane at the quinone B binding site), stopping the flow of electrons and causing the accumulation of free radicals (Friesen and Powles, 2007). Another atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine) binding site – protein D2 – was reported to exist, which partially inhibits the recombination reaction of ‘S’ charge states of the oxygen-evolving system (Jursinic et al., 1991). Because of the low affinity of this site, only small amounts of atrazine will bind to it, causing negligible effects and playing a secondary role in susceptible plants, in which the higher affinity site is that of protein D1 (Jursinic et al., 1991; Walsh et al., 2012).

Leaf chlorophyll content is important to estimate the photosynthetic potential, as it is responsible for light energy absorption and transfer. Changes in physiological parameters of plants exposed to stress (e.g., to herbicides) can be detected by certain methods, such as chlorophyll *a* fluorescence and gas exchange measurements (Sousa et al., 2014; Kalaji et al., 2016). Chlorophyll *a* fluorescence provides information about pigment complexes, their organization, excitation energy transfer between them, and several PSII-specific electron transfer reactions (Stirbet and Govindjee, 2011). Thus, damages to the photosynthetic apparatus can be detected, even when the symptom is not visible yet (Giroto et al., 2010).

Therefore, knowledge of the primary metabolism in resistant plants exposed or not to atrazine herbicide is crucial to assess the occurrence of plant damage as a result of herbicide application. For this reason, the aim of this study was to investigate the action of atrazine on photochemical and biochemical processes of photosynthesis in triazine-resistant canola plants.

## MATERIAL AND METHODS

The experiment was performed in a greenhouse, at the Embrapa Trigo, Passo Fundo, RS, Brazil, using canola hybrid Hyola® 555TT, which is resistant to triazine herbicides, in a randomized block design with three replications. The greenhouse was set to the temperature of 20 °C, with natural lightning (approx. 13:40 hours of photoperiod). Three plants were sown in 4.8 liter pots filled with peat substrate (Plantas Garden Plus Turfa Fértil), and each pot was regarded as an experimental unit. Irrigation was performed manually, every two days, in the trays in which the pots had been placed, maintaining a water depth of approximately 2 cm.

Atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine) was applied at the dose of 2 kg of active ingredient per ha<sup>-1</sup>, when canola plants were at the four-leaf stage, using a CO<sub>2</sub> backpack sprayer with a spraying volume of 120 L ha<sup>-1</sup>. Treatments consisted of the application or not (control) of atrazine. The evaluations were performed at one, three, five, and eight days after application (DAA).

### Chlorophyll indexes

Chlorophyll *a* and *b* and total chlorophyll indexes were estimated using a chlorophyll meter (Falker, model ClorofiLog) in three leaves of each plant, in a total of 27 measurements per

treatment, with values expressed as Falker chlorophyll index (FCI). FCI is a relative chlorophyll content value based on absorbance and reflectance correlations. The chlorophyll *a/b* ratio was also calculated.

### Modulated chlorophyll *a* fluorescence

A fluorescence chamber coupled to an infrared gas meter (LI-COR, model LI-6400-XT) was used to assess modulated fluorescence. The measurements were performed according to the methodology proposed by Sousa et al. (2014) with modifications. Three measurements were performed per treatment, on completely expanded leaves. The leaves were stored in the dark for 20 min. After that, modulated light was emitted at  $0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  at 0.25 kHz in order to estimate initial fluorescence ( $F_0$ ). Maximal fluorescence ( $F_M$ ) was calculated after a saturating light pulse of  $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Thereafter, actinic lighting (light absorbed by the photosynthetic apparatus which causes the transport of electrons) was provided with a photosynthetically active radiation of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  for stabilization of photosynthetic rates. Afterwards, a sequence of 20 pulses of saturating light ( $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was applied at 30-second intervals. During each pulse, the following parameters were obtained or calculated:  $F_M'$  (maximal fluorescence in a light-adapted state);  $F_0'$  (minimal fluorescence measured immediately after actinic light was switched off);  $F_S$  (fluorescence obtained in a steady-state or terminal fluorescence);  $F_V/F_M$  (maximum photochemical efficiency) [ $F_V/F_M = (F_M - F_0)/F_M$ ];  $F_V'/F_M'$  (actual maximum photochemical efficiency) [ $F_V'/F_M' = (F_M' - F_0')/F_M'$ ];  $\Phi_{\text{PSII}}$  (quantum efficiency of PSII) [ $\Phi_{\text{PSII}} = (F_M' - F_S)/F_M'$ ]; ETR (electron transport rate) [ $\text{ETR} = \Phi_{\text{PSII}} \cdot f \cdot I \cdot \hat{a}_{\text{leaf}}$ ], wherein  $\Phi_{\text{PSII}}$  corresponds to the quantum efficiency of PSII,  $f$ , the fraction of absorbed photons that are used by PSII, set as 0.50 in this study,  $I$ , incident photon flux density ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and  $\hat{a}_{\text{leaf}}$ , leaf absorbance, set as 0.84;  $qP$  (photochemical quenching) [ $qP = (F_M' - F_S)/(F_M' - F_0')$ ];  $qN$  (non-photochemical quenching) [ $qN = (F_M - F_M')/(F_M - F_0)$ ]; and NPQ (non-photochemical quenching) [ $\text{NPQ} = (F_M - F_M')/F_M'$ ] (Baker, 2008; Sousa et al., 2014).

### Gas exchange

Stomatal conductance ( $g_s$ ), net carbon assimilation rate ( $A_L$ ), and transpiration ( $E$ ) were measured using the largest leaf of two plants from each pot, in a total of six measurements per treatment. The measurements were performed using an infrared gas meter, with a photosynthetic photon flux density of  $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ , in a growth chamber (LI-COR, model LI-6400-2B), at an atmospheric  $\text{CO}_2$  concentration (400 parts-per-million). Water-use efficiency (WUE) was also calculated [ $\text{WUE} = A_L/E$ ].

### Statistical analysis

The standard deviation of the measurements was used to compare the means of the treatments for the fluorescence variables evaluated during emission of saturating light pulses. Analysis of variance ( $p \leq 0.05$ ) was performed for the other variables and, when there was statistical significance, Tukey's test ( $p \leq 0.05$ ) was used to compare the means, considering both interactions within each assessment period and the mean of assessment periods in the absence of interaction.

## RESULTS AND DISCUSSION

### Chlorophyll indexes

No interaction was found among the factors (application of atrazine and assessment periods) associated with chlorophyll index. However, a separate effect of treatments was detected, showing stronger effects for all variables when atrazine was used (Table 1). Chlorophyll *a* and *b* and total chlorophyll indexes were higher, yielding 4.21, 10.89, and 5.51% in atrazine treatments.

The structure of the chlorophyll molecule has four nitrogen (N) atoms; therefore, higher amounts of this molecule may be associated with an increase in nitrogen metabolism in the

**Table 1** - Chlorophyll *a* and *b*, chlorophyll *a/b* ratio and total chlorophyll indexes in Hyola® 555TT canola hybrid for treatments with and without atrazine with means for assessment periods

Treatment	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll <i>a/b</i>	Total Chlorophyll
With atrazine	26.99 a	6.72 a	4.04 b	33.72 a
Without atrazine	25.90 b	6.06 b	4.30 a	31.96 b
CV (%)	4.01	6.82	3.41	4.40

Means followed by different letters in the column are statistically different according to Tukey's test ( $p \leq 0.05$ ).

plant. Some reports indicate an increase in chlorophyll content in corn after application of atrazine (Rao, 1982). The use of simazine (6-chloro-N<sub>2</sub>,N<sub>4</sub>-diethyl-1,3,5-triazine-2,4-diamine), a PSII inhibitor from the triazine group, boosted the growth and nitrogen content in peach (*Prunus persica* (L.) Batsch) and apple (*Malus domestica* Borkh.) (Ries et al., 1963). These effects could result from the increase in nitrate reductase activity or from auxin-like activity (Ries et al., 1967; Copping et al., 1972). An increase in the activity of this enzyme has been described in rye after simazine application, indicating that this herbicide can elevate nitrate uptake or increase enzyme synthesis or activation (Ries et al., 1967). Nitrate reductase reduces nitrate (NO<sub>3</sub><sup>-</sup>), taken up by the roots, to nitrite (NO<sub>2</sub><sup>-</sup>). Later, nitrite is converted by nitrite reductase to ammonia (NH<sub>3</sub><sup>+</sup>), which is then converted into amino acids and proteins (Taiz and Zeiger, 2013).

There are some reports on higher chlorophyll contents in atrazine-treated plants, which is known as 'greening' effect (Ebert and Dumford, 1976). An increase in total chlorophyll content was described for atrazine-treated barley (*Hordeum vulgare* L.) leaves, which the authors attributed to a stimulatory effect of the herbicide on the enzyme involved in chlorophyll biosynthesis (Klendgen, 1979 apud Flora et al., 2013). In a study carried out with peas treated with triazine herbicides, there was an increase in the activity of aminolevulinic acid dehydratase, which acts in the biosynthesis of porphyrins, heme compounds and chlorophyll (Wu et al., 1971).

The chlorophyll *a/b* ratio was lower in plants treated with atrazine, because of a considerable increase of chlorophyll *b* in relative terms (4.2% of increase in chlorophyll *a*; 10.9% of increase in chlorophyll *b*). When the total chlorophyll index remains unchanged and the chlorophyll *a/b* ratio decreases, it is said that the plant adapted to less light energy intensity (shade), which points to lower capacity of plants to deal with excessive light energy (Rensen and Vredenberg, 2011).

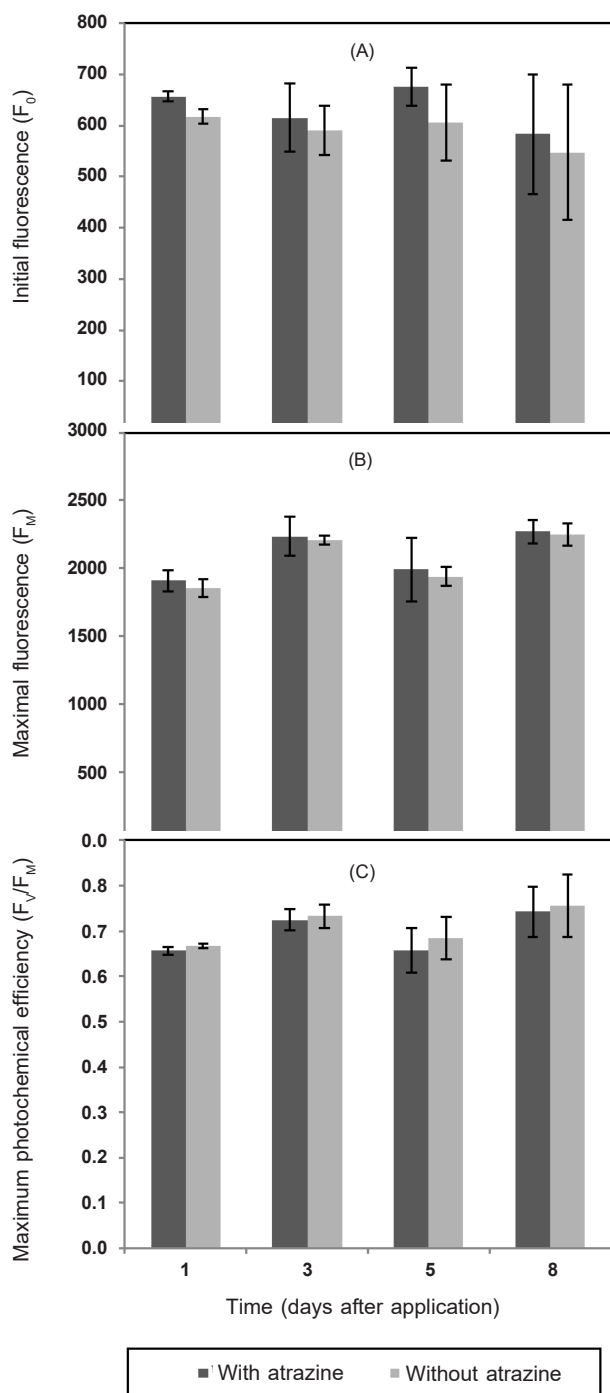
However, given that the total chlorophyll index also increased in response to atrazine in resistant canola plants (Table 1), the lower chlorophyll *a/b* ratio will not cause damage to plants exposed to excessive light. Therefore, it cannot be said that atrazine-resistant canola plants are more sensitive to photoinhibition when exposed to this herbicide as both chlorophyll *a* and *b* indexes increased, allowing plants to take up more light energy.

### Modulated chlorophyll *a* fluorescence

With respect to  $F_0$ ,  $F_0'$ ,  $F_M$  and  $F_M'$ , the modulated fluorescence of chlorophyll *a* showed no interaction between factors and no differences between treatments (with and without atrazine) (Figure 1 and Figure 2). Initial fluorescence ( $F_0$  and  $F_0'$ ) was measured using oxidized  $Q_A$  and maximal fluorescence ( $F_M$  and  $F_M'$ ) using reduced  $Q_A$ . However, in dark-adapted leaves, from which parameters  $F_0$  and  $F_M$  are obtained, non-photochemical quenching is inactive; in comparison, in light-adapted leaves, from which parameters  $F_0'$  and  $F_M'$  are obtained, quenching is active (Koblížek et al., 2001).

Steady-state fluorescence –  $F_s$  – was different for treatments at 1 DAA (Figure 2A), in which a higher  $F_s$  was found with the atrazine treatment. In other assessment periods, this difference decreased, and there was no difference between treatments (Figures 2B to 2D).

$F_s$  corresponds to the energy released by the electrons that moved beyond the quinone quencher in the electron transport chain, on the path to photosystem I, i.e., it points to the loss of energy by electrons between photosystems II and I (Vieira et al., 2010). Therefore, it may be considered that the application of atrazine in resistant canola plants plays a role at this stage of



Vertical bars indicate standard deviations.

**Figure 1** - Initial fluorescence ( $F_0$ ) (A) and maximal fluorescence ( $F_M$ ) (B) for chlorophyll *a* and maximum photochemical efficiency ( $F_V/F_M$ ) (C) in Hyola® 555TT canola hybrid for treatments with and without atrazine at one, three, five, and eight days after application.

quenching, which represents the excitation energy lost in the form of heat, was not influenced by the application of atrazine in resistant canola plants.

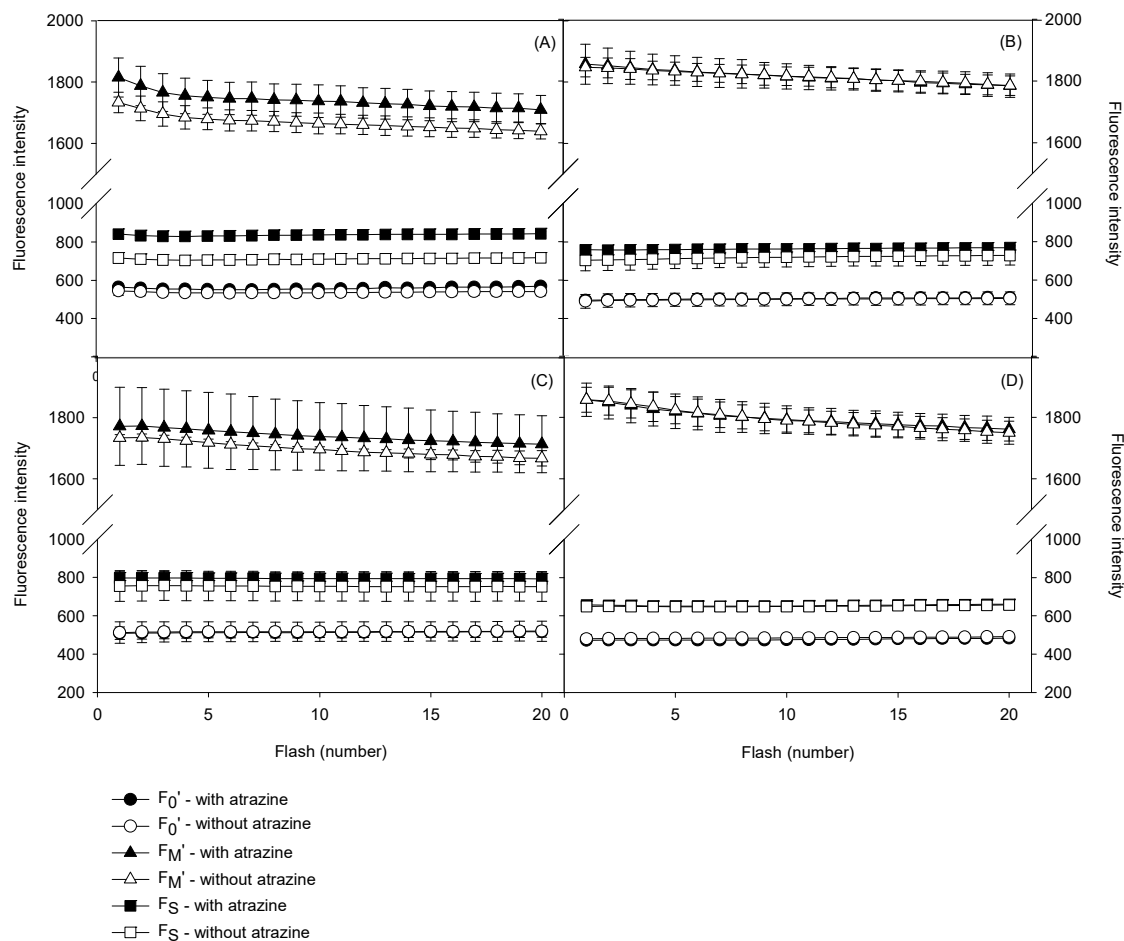
$F_V'/F_M'$  ranged from 0.65 to 0.75 for plants not exposed to atrazine treatment, in all light pulses (Figure 3). Low values of  $F_V'/F_M'$  are expected for hybrid Hyola® 555TT, since the mechanism of resistance to triazines in this hybrid leads to a smaller PSII activity (Rensen and Vredenberg, 2011). The mutant D1 protein causes charge recombination in PSII instead of the use of electrons

the electron transport chain, interfering with the transport of electrons between photosystems II and I.

Initial fluorescence ( $F_0$ ) represents the energy released by chlorophyll *a* molecules from the antenna of PSII before the electrons migrate to the reaction center. Thus, there may be changes in  $F_0$  when the transfer of excitation energy from the light collecting system to the reaction center is reduced or when there is damage to the reaction center (Mathis and Pallotin, 1981; Baker and Rosenqvist, 2004).  $F_M$  indicates the energy released by electrons that flow back to their original molecule because of the presence of some electron flow blocker or lack of demand for nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH), adenosine triphosphate (ATP) or ferredoxin production (Mathis and Pallotin, 1981; Baker, 2008). Initial and maximal fluorescence, both in dark- and light-adapted leaves, did not show statistical differences between treatments, which indicates that the application of atrazine in resistant canola plants does not hinder the transfer of excitation energy to the reaction center nor blocks the flow of electrons (Figure 1 and Figure 2).

Regarding maximum photochemical efficiency –  $F_V/F_M$  – and actual photochemical efficiency –  $F_V'/F_M'$  –, there were no differences between treatments (Figure 1 and Figure 3). Several authors concluded that  $F_V/F_M$  is not a good indicator of stress in the photosynthetic apparatus, as there were no differences between the control treatment and several stressors (Mehta et al., 2010; Sousa et al., 2014). In the present study,  $F_V/F_M$  ranged from 0.66 to 0.76 (Figure 1). These values are lower than those regarded as normal (0.75 to 0.85) for plants not subjected to stress (Bolhar-Nordenkamp et al., 1989).

Actual photochemical efficiency ( $F_V'/F_M'$ ) is obtained from  $F_0'$  and  $F_M'$  measurements made when non-photochemical quenching is active. Hence, this parameter is useful for evaluating the contribution of non-photochemical quenching to changes in the operating efficiency of PSII in light-adapted leaves (Baker, 2008). Therefore, non-photochemical



Vertical bars indicate standard deviations.

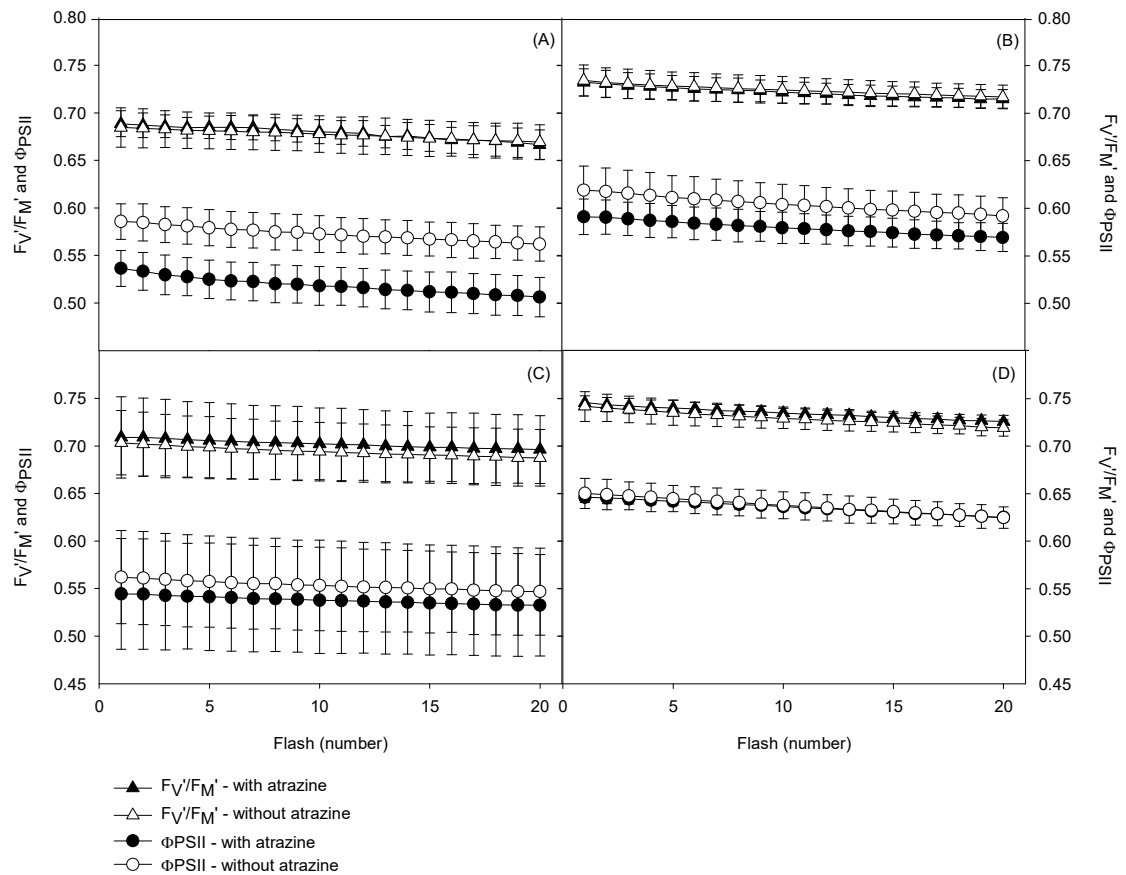
**Figure 2** - Initial fluorescence ( $F_0'$ ), maximal fluorescence ( $F_M'$ ), and steady-state fluorescence ( $F_S$ ) for chlorophyll *a* in Hyola® 555TT canola hybrid for treatments with and without atrazine and light flashes at one (A), three (B), five (C), and eight (D) days after application.

in linear transport, which would reduce nicotinamide adenine dinucleotide phosphate in the oxidized form ( $NADP^+$ ) and generate ATP, for later use in carbon reduction reactions (Fuerst and Norman, 1991).

Quantum efficiency of PSII –  $\Phi_{PSII}$  – was different for treatments at 1 DAA (Figure 3A). Higher  $\Phi_{PSII}$  was found in the atrazine-free treatment. This may be explained by the estimation method, which relies on  $F_S$  values; it also differed between treatments in this assessment period. Therefore, an increase in steady-state fluorescence in atrazine-treated plants led to lower quantum efficiency of PSII. In other periods, the differences between treatments decreased and were no longer significant (Figure 3B to 3D).

$\Phi_{PSII}$  measures the rate of light absorbed by PSII-associated chlorophyll used in the photochemical process and may provide an estimate of the linear electron transport rate and of total photosynthesis (Maxuell and Johnson, 2000). When there are changes in this parameter, net  $CO_2$  assimilation rates are also expected to differ.

Corroborating the results obtained for  $\Phi_{PSII}$ , the electron transport rate – ETR – was lower for the treatment without atrazine, at 1 DAA, while in the other periods, treatments did not differ (Figure 4). The ETR in treatments with and without atrazine was, respectively, 18.2 and 19.2 at 1 DAA, 23.1 and 24.1 at 3 DAA, 21.3 and 22.1 at 5 DAA, and 25.1 and 25.2 at 8 DAA, on the average of light pulses (data not shown). Such values correspond to a reduction in ETR, as a result of atrazine application, of 5.5, 4.3, 3.5, and 0.1% for the evaluation periods of 1, 3, 5, and 8 DAA, respectively.



Vertical bars indicate standard deviations.

**Figure 3** - Actual maximum photochemical efficiency ( $F_v'/F_M'$ ) and quantum efficiency of photosystem II ( $\Phi_{PSII}$ ) in Hyola<sup>®</sup> 555TT canola hybrid for treatments with and without atrazine and light flashes at one (A), three (B), five (C), and eight (D) days after application.

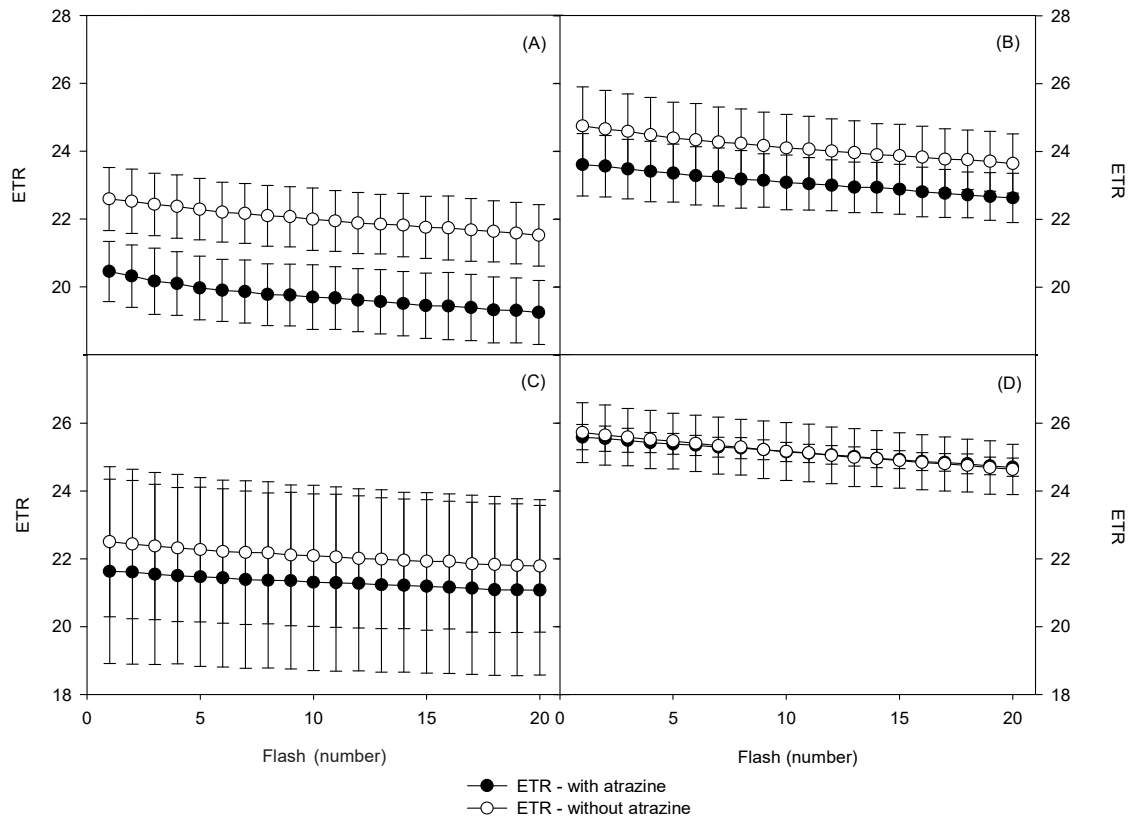
Both  $q_N$  and NPQ did not show differences between treatments in any of the assessment periods (Figure 5A to 5D). Non-photochemical quenching (NPQ) is a photoprotective process that removes excess excitation energy within chlorophyll-containing complexes by means of heat dissipation, preventing the formation of damaging free radicals (Murchie and Lawson, 2013). This process is regulated by a protein, PsbS, and the xanthophyll cycle (Kiss et al., 2008; Murchie and Niyogi, 2011; Ruban et al., 2012).

Acidification of the thylakoid lumen leads to protonation of PsbS and formation of zeaxanthin, changing the conformation of the PSII antenna, thereby inducing quenching of excitation energy (Ruban et al., 2012). Because NPQ requires knowledge of the dark-adapted values ( $F_0$  and  $F_M$ ), if the value of  $F_v'/F_M'$  is lower than 0.83, which occurred in the present study, the NPQ value should be treated with caution, especially when comparing leaves with different  $F_v'/F_M'$  values (Murchie and Lawson, 2013).

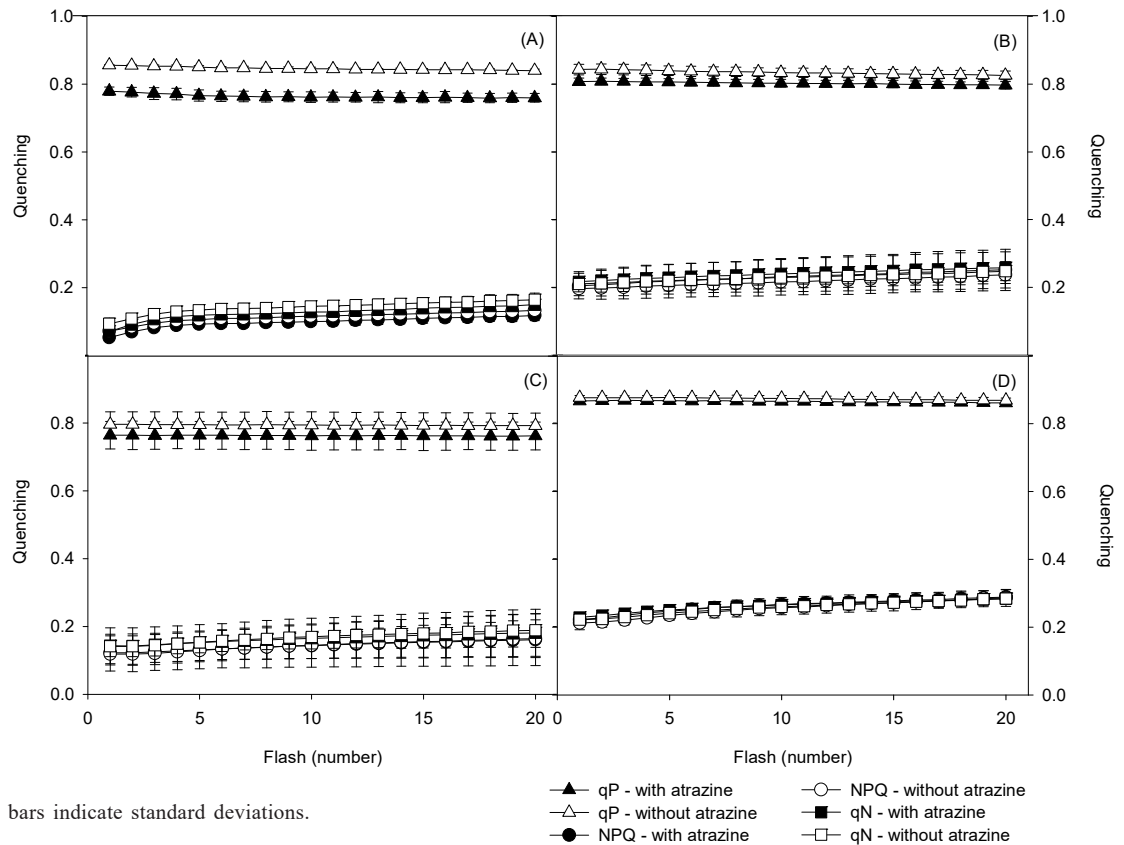
Photochemical quenching ( $q_P$ ) differed at 1 and 3 DAA and was higher in the atrazine-free treatment for all light pulses (Figure 5A and 5B). Again, the influence of  $F_s$  on the other calculated parameters is perceptible. Photochemical quenching uses  $F_s$  for its estimation while non-photochemical quenching ( $q_N$  and NPQ) does not take this variable into consideration. The level of photochemical quenching of PSII indicates the proportion of reaction centers that are open, in a non-linear relationship (Murchie and Lawson, 2013).

### Gas exchange

Regarding gas exchange at 1 DAA, there were differences between treatments for the variable  $g_s$  (Figure 6A). The atrazine-free treatment showed higher stomatal conductance, outperforming



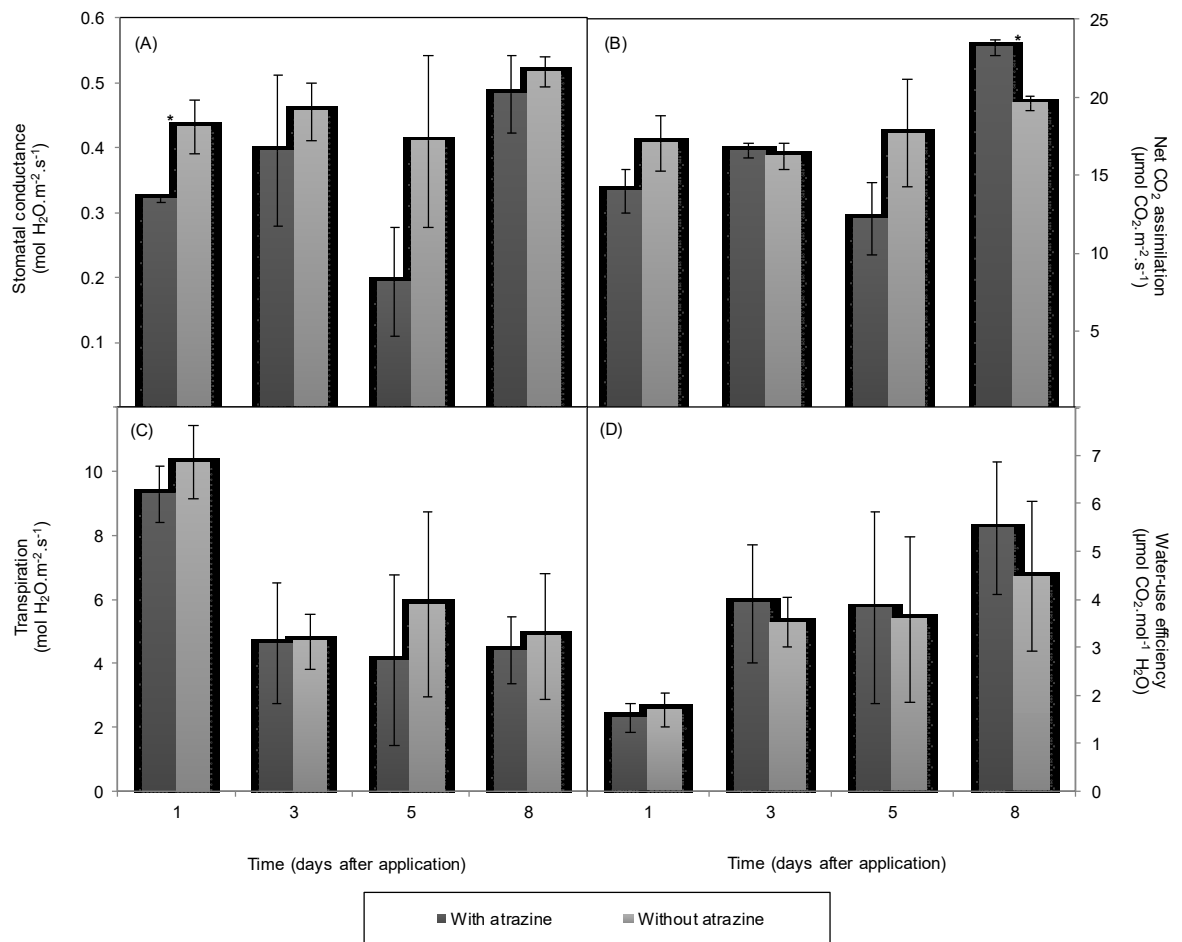
**Figure 4** - Electron transport rate (ETR) in Hyola® 555TT canola hybrid for treatments with and without atrazine and light flashes at one (A), three (B), five (C), and eight (D) days after application.



Vertical bars indicate standard deviations.

**Figure 5** - Photochemical quenching (qP) and non-photochemical quenching (NPQ and qN) in Hyola® 555TT canola hybrid for treatments with and without atrazine and light flashes at one (A), three (B), five (C), and eight (D) days after application.





\* Significant difference between treatments, according to Tukey's test ( $p \leq 0.05$ ). Vertical bars indicate standard deviations.

**Figure 6** - Stomatal conductance (A), net CO<sub>2</sub> assimilation (B), transpiration (C), and water-use efficiency (D) in Hyolra® 555TT canola hybrid for treatments with and without atrazine at one, three, five, and eight days after application.

the other treatment by 34.37%. The increase in stomatal conductance leads to higher CO<sub>2</sub> diffusion within leaves, thereby increasing the photosynthetic rate, thus possibly augmenting biomass accumulation and yield (Taiz and Zeiger, 2013).

Despite the difference found in  $g_s$ , there was no influence on  $A_L$  at 1 DAA and statistical differences between treatments were not detected, even though higher rates were found for the atrazine-free treatment (Figure 6B). The higher stomatal conductance in plants not subjected to atrazine treatment was detected only at 1 DAA; thus, it may not have been sufficient to change  $A_L$ . At 3 and 5 DAA, treatments did not differ for the assessed gas exchange parameters, indicating that the effect of atrazine on stomatal conductance did not last and did not interfere with the other primary metabolism parameters (Figure 6).

Plants treated with atrazine showed a higher  $A_L$  value at 8 DAA, which was 18.37% higher than in the atrazine-free treatment (Figure 6B). This increase in the photosynthetic rate in plants treated may demonstrate recovery of the plant from the stresses caused by the herbicide or be a result of the increase in chlorophyll indexes.

Atrazine increased chlorophyll indexes in resistant canola plants, which may be the result of an increase in nitrate reductase activity or of the stimulatory effect of the herbicide on the enzyme responsible for chlorophyll biosynthesis. As observed in chlorophyll *a* fluorescence, atrazine increased steady-state fluorescence and reduced quantum efficiency of PSII and electron transport rate at 1 DAA, in addition to reducing photochemical quenching at 1 and 3 DAA (Figures 2 to 5). These results indicate the interference of atrazine in electron transport between the photosystems, even though the hybrid is resistant to this herbicide.

The higher net carbon assimilation rate at 8 DAA in plants exposed to atrazine treatment may be explained by the higher chlorophyll indexes in these plants in all assessment periods. Higher chlorophyll indexes improve the capacity of plants to take up light energy which is later used to produce ATP and NADPH - potent reducing agents which are crucial for carbon reduction reactions.

In this paper, the authors reported the effects of atrazine application on photochemical and biochemical processes in a resistant canola hybrid. In another work evaluating more herbicides and canola hybrids, in a greenhouse, non-treated plants of the hybrid Hyola® 555TT presented 20% more shoot dry matter than those that received the treatment with atrazine (2 kg i.a. ha<sup>-1</sup>), at 29 days after application, despite no differences were found according to the *t* test (*p*=0.07) (authors, data not published).

In conclusion, the application of atrazine temporarily reduces electron transport between photosystems II and I and increases chlorophyll indexes in resistant canola plants, raising the net carbon assimilation rate at eight days after treatment. More studies are needed to clarify the effects of atrazine on dry matter production at maturity and grain yield by triazine-resistant canola plants, as well as evaluate other triazine-resistant canola hybrids.

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