



## Article

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## INTOXICATION AND PHYSIOLOGICAL ASPECTS OF FORAGE PLANTS AND WEEDS SUBMITTED TO CLOMAZONE ATMOSPHERIC WASTE

*Intoxicação e Aspectos Fisiológicos de Plantas Forrageiras e Daninhas Submetidas a Resíduos Atmosféricos de Clomazone*

**ABSTRACT** - Herbicide volatilization may generate environmental and agricultural problems and result in visual or physiological contamination of non-target plant species. Thus, the goal of this research was to study the fluorescence of chlorophyll a in weeds and fodder plants under the effect of clomazone in the form of atmospheric waste. The experiment was conducted under field conditions designed in randomized blocks with four replications, in a 6 x 4 factor scheme, with six plant species: *Dolichos lablab*, *bicolor Sorghum*, *Urochloa brizantha*, *Macrotyloma axillare*, *Portulaca oleracea* and *Sida rhombifolia*. There were four solutions containing 0, 360, 720 and 1,080 g ha<sup>-1</sup> of clomazone (0, 0.05, 0.10 and 0.15 mg L<sup>-1</sup>, considered as the volume). Seedbeds were built and covered with transparent polyethylene film of 150 µm, with a volume of 12 m<sup>3</sup>. Fodder plants were sown in line, while weeds were selected according to the incidence. On the sixteenth day after emergence, concentrations of herbicide diluted on three petri dishes were inserted. After 72 hours of exposure, the tunnels were opened and the dishes were removed, noticing evaporation of the product. The following evaluations were performed: plant poisoning, initial fluorescence, maximum quantum yield of PSII, photochemical quenching, non-photochemical quenching and chlorophyll content. Even at concentrations that do not promote visual effect, clomazone can cause significant damage in the photosynthetic activity of the species. The physiological variables chlorophyll, maximum quantum yield of PSII and initial chlorophyll fluorescence can be effectively used to monitor clomazone waste in the atmosphere.

**Keywords:** *Dolichos lablab* L., *Sorghum bicolor* L. Moench, *Urochloa brizantha* Stapf, *Macrotyloma axillare* E. Mey, shut tunnels.

**RESUMO** - A volatilização de herbicidas pode gerar problemas ambientais e agrícolas e resultar em contaminação visual ou fisiológica de espécies vegetais não alvo. Assim, objetivou-se com esta pesquisa estudar a fluorescência da clorofila a de plantas daninhas e forrageiras sob efeito do clomazone na forma de resíduos atmosféricos. O experimento foi conduzido em campo, delineado em blocos casualizados com quatro repetições, em esquema fatorial 6 x 4, sendo seis espécies vegetais: *Dolichos lablab*, *Sorghum bicolor*, *Urochloa brizantha*, *Macrotyloma axillare*, *Portulaca oleracea* e *Sida rhombifolia*, em quatro soluções contendo clomazone: 0, 360, 720 e 1.080 g ha<sup>-1</sup> (0, 0,05, 0,10 e 0,15 mg L<sup>-1</sup>). Foram construídos canteiros, cobertos com filme de polietileno transparente de 150 µm, apresentando volume de 12 m<sup>3</sup>. As forrageiras foram semeadas em linha, enquanto as plantas daninhas foram selecionadas conforme a incidência no local do experimento. No décimo sexto dia após a emergência, foi inserida a concentração do herbicida

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*diluído em três placas de Petri. Transcorridas 72 horas de exposição, os túneis foram abertos e as placas retiradas, constatando-se evaporação do produto. Procedeu-se às seguintes avaliações: intoxicação das plantas, fluorescência inicial, rendimento quântico máximo do PSII, quenching fotoquímico, quenching não fotoquímico e teor de clorofila. As variáveis fisiológicas, clorofila total, rendimento quântico máximo do PSII e fluorescência inicial da clorofila podem ser usadas de forma eficiente no monitoramento de resíduos do clomazone na atmosfera. Mesmo em concentrações que não promovem efeito visual, o clomazone é capaz de causar danos na atividade fotossintética das espécies.*

**Palavras-chave:** *Dolichos lablab* L., *Sorghum bicolor* L. Moench, *Urochloa brizantha* Stapf, *Macrotyloma axillare* E. Mey, túneis fechados.

## INTRODUCTION

A large part of the herbicides applied to control weeds tends to move into soil, water and also the atmosphere, whereas the other part is absorbed by plants (Mancuso et al., 2011). When in the atmosphere, the product is carried for long distances and deposited on non-target organisms. Thus, studies about the loss of these products in the atmosphere are important to evaluate the risk that they may trigger over non-target species, such as people, animals and other vegetal species (Ellis et al., 2010).

Brazil is the greater producer of food, fibers and energy in the world; therefore, the use of pesticides tends to be high. However, when observing area units, this value tends to be low. Thus, due to the continental surface of the country, the final applied quantity is quite high. According to the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária), since 2009 Brazil is the greatest consumer and producer of pesticides in the world. The market of these products grew approximately 176% over the last decade; it is four times bigger than the world average (ANVISA, 2015).

Each herbicide molecule presents its own characteristics and, when in contact with the environment, it tends to have reactions. Among the main processes involved after the application of herbicides, there is retention, transformation and transport, which encompass drifting, volatilization, leaching and superficial runoff (Mancuso et al., 2011).

Among the main available molecules to control weeds, clomazone 2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone) stands out because it controls well various weed species. It is indicated for various cultures, including cotton, rice, tobacco, sugarcane, cassava and soybean (Brazil, 2016).

In spite of being safe as for waste on food and plants, clomazone dissipation way, via solubility in water and volatilization, makes its waste prone to promoting visual toxic effect. Volatilization represents the global result of all physical-chemical processes through which the compound is transferred from soil and/or plant surface into the atmosphere (Bedos et al., 2002). Clomazone may be lost in the environment through drifting. Drifting is undesirable because of the direct damages at economic level and damages to the adjacent sensitive cultures, as well as causing contamination to food, air and water resources (Ozkan, 2000). In many cases, the product is transported to another destination, reaching non-target plants. In this scenario, fodder or leafy plant species behave as susceptible, because many production areas are located next to pastures or big cultivations (Rigoli et al., 2008).

Clomazone acts within the isoxazolidinone group and is classified as an inhibitor of carotenoid biosynthesis. With the herbicide action, there will be inhibition of carotenoid synthesis and, without it, chlorophyll will be degraded through photo-inhibition, reducing the photosynthetic potential. With the destruction of chlorophyll, there will be depigmentation or whitening symptoms, until reducing the growth of sensitive plant species (Ferhatoglu et al., 2006; Senseman, 2007).

In spite of the already researched effects of waste into soil over non-target plants, little is known about the behavior of the different species in relation to the atmospheric waste of clomazone and which atmospheric concentration of this product is capable of causing visual toxic effect on sensitive plants. In various situations, the damages caused by herbicide intoxication

are not visually noticeable, in some cases due to the evaluation periods. Thus, some authors have proposed to evaluate the physiological responses of cultures as a way to select herbicide with application potential (Galon et al., 2010; Torres et al., 2012).

In order to determine the physiological effects of using herbicides, several equipments have been used, and the fluorescence parameter has been widespread mainly in studies on the effects at photosynthetic levels, which allow analyzing quantitatively and qualitatively the absorption and use of light energy through the photosystem II, and the possible relations with the photosynthetic capacity (Torres Neto et al., 2005; Ferraz et al., 2014). In this regard, the study on the physiology of different plants when exposed to low concentrations may help monitoring and prevent environmental problems involving herbicides. This research was proposed with the goal to study the physiology of weeds and fodder plants under the effects of clomazone in the form of atmospheric waste.

## MATERIAL AND METHODS

The experiment was conducted in an experimental field, from September to December 2015. Different concentrations of clomazone were estimated on the physiological activity of fodder plants and weeds. The randomized block design with four replications was used, in a 6 x 4 factor scheme; the first factor consisted in six plant species [four fodder plants: hyacinthbean (*Dolichos lablab*), sorghum (*Sorghum bicolor*), palisade grass (*Urochloa brizantha*) and perennial horsegram (*Macrotyloma axillare*) and two weeds: purslane (*Portulaca oleracea*) and arrowleaf sida (*Sida rhombifolia*)], and the second one consisted in four clomazone solutions: 0, 360, 720 and 1,080 g ha<sup>-1</sup> (0, 0.05, 0.10 and 0.15 mg L<sup>-1</sup>).

The GAMIT 360® formula was used, with 36% clomazone. The species were sown in previously fertilized seedbeds and with no recent history of herbicide application. The chemical analysis of soil presented the following composition: water pH (1:2.5) = 5.0; CTC pH7 = 9.17 cmol<sub>c</sub> dm<sup>-3</sup>; MO = 2.3 dag dm<sup>-3</sup> Ca = 1.5 cmol<sub>c</sub> dm<sup>-3</sup>; Mg = 0.5 cmol<sub>c</sub> dm<sup>-3</sup>; exchangeable Al = 0.54 cmol<sub>c</sub> dm<sup>-3</sup>; available P = 4.3 mg dm<sup>-3</sup>; exchangeable K = 29 mg dm<sup>3</sup>; V = 23%; and sandy texture, being classified as Quartzarenic Neosol (Entisol).

In order to prepare the soil, the area was submitted to plowing and harrowing; subsequently, 6.00 m long and 1.60 m wide seedbeds were prepared. Soil was submitted to liming, applying 2.0 ton ha<sup>-1</sup> of Dolomitic limestone, and it was later fertilized with 2,220, 206 and 363 kg ha<sup>-1</sup> of phosphorus, potassium and nitrogen, respectively.

During the construction of the plastic tunnels, 50 cm long pile lines were distributed along the sides of the seedbeds, spaced two meters apart, where 2.50 m long arches were tied. The seedbeds were covered with 150 m of transparent low density polyethylene film; the inside of the tunnel presented and approximate volume of 12 m<sup>3</sup> (Figure 1). Tunnels were spaced 2.00 m apart. The ends of the polyethylene film were tied on piles at the top and bottom of the tunnels, and they were covered in soil to avoid the loss of gases into the external environment.

Fodder plants' sowing was performed in lines, using ten seeds from each species per meter; for each species, four meters were selected, which were spaced 15 cm between the lines. Plants were spaced 10 cm apart. On the other hand, weeds were selected according to germination and incidence in the experimental area of the cultivation; the ones appearing in all seedbeds were used. The performed cultural treatments had the goal to prevent the competition between the

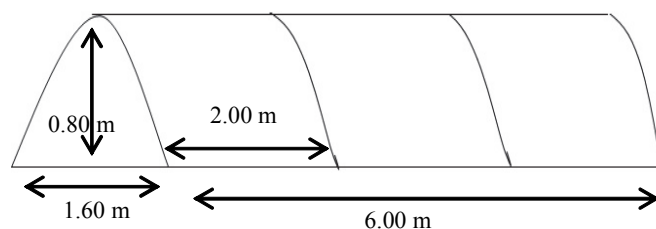


Figure 1 - Arrangement and size of each experimental unit for the cultivation of fodder plants.

fodder plants and weeds that were selected for the test. Irrigation was performed with the goal to keep field capacity. A micro-perforated hose for localized irrigation was used, and the adopted irrigation interval was twice a day. Temperature inside the tunnels was monitored during the research, presenting an average of 35 °C ( $\pm 7$  °C).

On day 16 after germination, three Petri dishes were placed inside each tunnel randomly, with clomazone doses corresponding to the treatment. Doses were diluted in de-ionized water. Tunnels were immediately closed, without any gas exchange with the external environment, and remained closed for three days, to allow the proper diffusion of the herbicide vapor. The use of Petri dishes had the goal to avoid the root absorption and direct loss of the product into the soil. After 72 hours of exposure, the tunnels were opened and the dishes removed, observing the complete evaporation of the solution. Effectiveness in the bio-indication of the presence of clomazone in the atmosphere was measured through a visual scale of plant clomazone intoxication, 16 days after the germination of the species, with a variation from 0 to 100%, where 0% corresponds to the absence of symptoms and 100% to the total death of the plant (SBCPD, 1995). For the effectiveness analysis of plant photosystem II, measurements were performed after 30 minutes of adaptation to darkness, with the emission of a saturating light pulse of 0.3 s, under a 0.6 KHz frequency by a fluorometer. Then, the initial fluorescence ( $F_0$ ), the maximum fluorescence ( $F_m$ ), the ratio between variable fluorescence and maximum fluorescence ( $F_v/F_m$ ), the photochemical quenching ( $q_P$ ) and the non-photochemical quenching ( $q_N$ ) and the electron transportation rate ( $ETR - \mu\text{Mols s}^{-1} \text{m}^{-2} \text{s}^{-1}$ ), as well as the total chlorophyll content, with a chlorophyll-meter, were evaluated.

Data were interpreted by analysis of variance  $p < 0.05$ ; when significant, they were submitted to regression at  $p < 0.05$  of error probability.

## RESULTS AND DISCUSSION

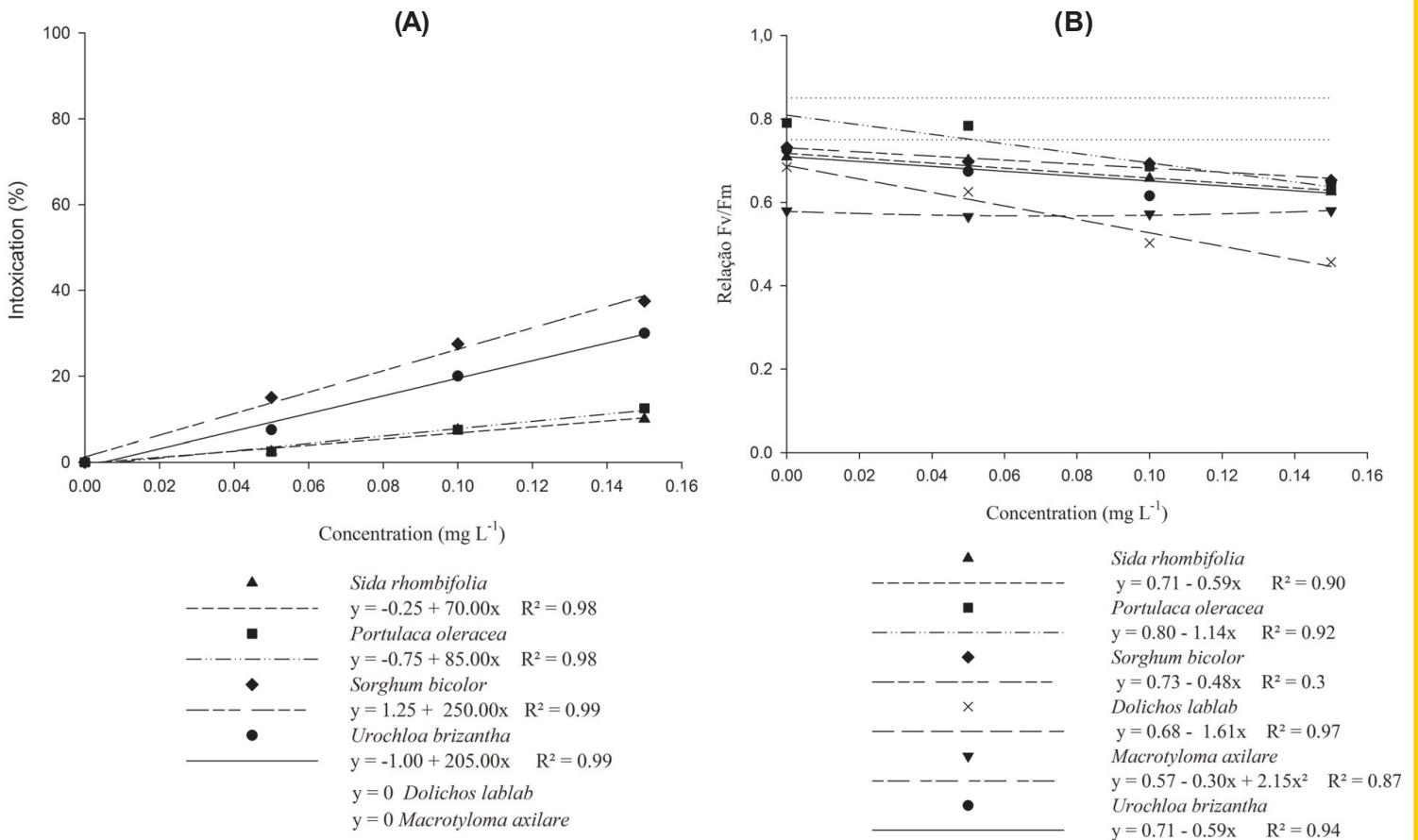
Concentrations of clomazone in the atmosphere caused intoxication in the species *Sida rhombifolia*, *Portulaca oleracea*, *Sorghum bicolor* and *Urochloa brizantha*. In these species, the yellowing and whitening of leaves were observed; they are typical intoxications caused by clomazone (Figure 2). It was observed that *S. bicolor* (sorghum) was the most sensitive to clomazone, with a 13.75% intoxication, followed by *U. brizantha*, *P. oleracea* and *S. rhombifolia*, which presented, under the effect of the minimum tested dose, visual intoxication lower than 10%. Among the species that demonstrated intoxication by the herbicide, it was possible to observe a gradual increase that was proportional to the increase in clomazone concentration, reaching values of 38.75, 29.75, and 10.25%, respectively for *S. bicolor*, *U. brizantha*, *S. rhombifolia* and *P. oleracea*, under the effect of the highest applied dose. The species *D. lablab* and *M. axillare* did not present any intoxication symptom with the increase in the concentration of clomazone (Figure 2A).

The absence of intoxication in some plants may be justified by development stage, morphology, absorption, translocation, environmental conditions, application period and metabolism, which are important factors that may determine the selectivity of the herbicide on plants (Santos et al., 2014). The selectivity of some plants to clomazone may also be related to the action site of the cytochrome P-450 mono-oxygenase enzyme, which is involved in resistance metabolites of various herbicides (Yun et al., 2001). This enzyme normally acts with the function of detoxifying, and it may be responsible for the oxidation of the clomazone molecule, making it more toxic to plants (Yun et al., 2005).

The activity of P450 cytochromes helps turning chemical products into extremely reactive products, maximizing by the oxidation of the molecule and making them more reactive. In this work, the powered effect of clomazone in the tested concentration was highlighted, with damages to species such as *S. bicolor* and *U. brizantha*. With these results, it is important to underline the importance of monitoring previously the presence of possible toxic molecules in the air, close to sensitive cultures.

The temperature during the experiment remained high, according to the microclimate resulting from the shape of the tunnels. This fact may have contributed to the higher plant intoxication, even in small clomazone concentrations. Schreiber et al. (2013) state that low temperatures cause less activity of the enzyme cytochrome P450, which will consequently affect





**Figura 2** - (A) Intoxication (%) of fodder plants and weeds under the effect of atmospheric clomazone; (B) maximum quantum yield of the PSII of fodder plants and weeds under the effect of atmospheric clomazone.

the activation of the herbicide and will cause lower intoxication. In this work, the high temperature may have powered the intoxication effect of clomazone.

The temperature increase may trigger the destabilization of the lipid bilayer, which may increase the fluidity and permeability of the membrane. Moreover, the oxidative stress may lead to the formation of toxic radicals, such as the reaction between O<sup>2-</sup> and hydrogen peroxide (Pastori and Trippi, 1993; Mendoza, 2014). The damage caused in this situation may become even worse: when thylakoid membranes are damaged, the acceptors that are located there are under the same effect, since there is no electron movement to PSII (Pastori and Trippi, 1993). In another research, it was verified that, within the photosynthetic system, PSII proved to be more sensitive to heat than PSI; enzymes from the stroma and the chloroplast envelope comparatively demonstrated to be more thermo-stable (Havaux and Davaud; 1994). For this reason, it is expected to have a reduction in the maximum quantum yield of the PSII, justifying the low values found in this work for the tested species.

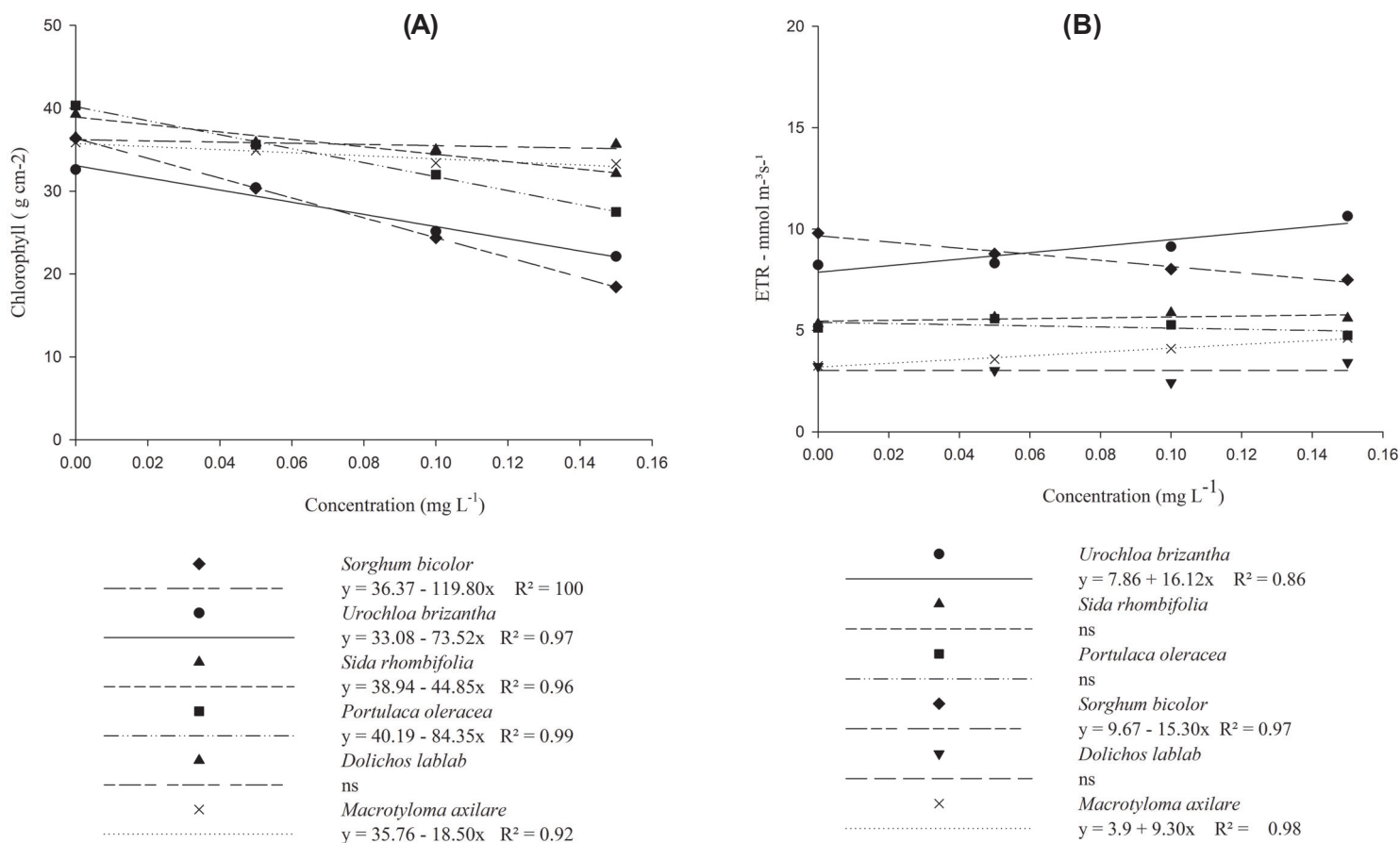
Considering that, for the maximum quantum yield of the PSII (Fv/Fm) of plants with an excellent physiological state, the Fv/Fm relation should vary between 0.75 and 0.85, it is possible to state that only *P. oleracea* remained within normality. This species presented a ration of 0.80; the value decreased with the increase in the clomazone concentration (Figure 2B). For the other species, even the ones that did not present visual toxicity symptoms, an effect in the Fv/Fm relation was observed. *D. lablab* stands out, decreasing from 0.68 to 0.45 with the increase in the concentration (Figure 2B). For the other species, it was possible to observe a slight reduction with the increase in the clomazone concentration; however, it was always lower than 0.75 in the sample treatment. Possibly, these low values are justified by the high temperatures recorded in the experiment location. Values above 0.85 may indicate a photo-inhibiting effect, when plants are submitted to chemical stress. Similarly, lower values indicate stress and reduction of

the maximum quantum effectiveness of the photosystem II and, consequently, reduction in the photosynthetic potential of the plant (Araus and Hogan, 1994; Ferreira et al., 2015).

Even with the low values of the sample treatment for Fv/Fm, it was possible to observe clomazone effects on the species, highlighting that, increasing the clomazone concentration, there are also effects on photosynthesis at a photo-chemical effect. An influence over photosynthesis must be expected, since the action mechanism of clomazone directly affects chlorophyll, which is responsible for the absorption of light energy.

There were effects from clomazone concentration over the chlorophyll content of the tested species, with the exception of *M. axillare*, that maintained average values between 30 and 35  $\mu\text{g cm}^{-2}$ . As for the other species, it is possible to notice an accentuated decrease in the chlorophyll content with the increase in the herbicide concentration, with an emphasis on *S. bicolor* and *P. oleracea*, reaching the concentration of 0.15  $\text{mg L}^{-1}$  with 18.43 and 27.5  $\mu\text{g cm}^{-2}$ , respectively (Figure 3A). It was possible to notice that the total chlorophyll content was more damaged than the photochemical effectiveness, which followed the intoxication degree of the species. This effect is possibly due to the fact that the herbicide molecule affects the formation of the chlorophyll pigment and not the transportation of electrons (Zera et al., 2011).

Since the specific action of clomazone occurs mainly by the inhibition of the deoxyxylulose phosphate synthase enzyme, which is responsible for the synthesis of isoterpenoids (which are basic precursors of carotenoids); the absence of carotenoids will directly affect the chlorophyll content (Ferhatoglu et al., 2006). With the destruction of chlorophyll by photo-oxidation, symptoms will appear firstly on the leaves of the tree tops, since clomazone is absorbed by the apical meristem. Clomazone does not act on already synthesized carotenoids; this justifies the presence of symptoms on the younger leaves, where the synthesis of carotenoids is concentrated (Ferhatoglu et al., 2006; Silva and Silva, 2007).



**Figura 3 -** (A) Total chlorophyll ( $\mu\text{g cm}^{-2}$ ) of fodder plants and weeds under the effect of atmospheric clomazone; (B) electron transportation rate (ETR -  $\text{mmol m}^{-3} \text{s}^{-1}$ ) of fodder plants and weeds under the effect of atmospheric clomazone.

Studies that evaluate the sensitivity of maize cultures to clomazone by the use of dietholate demonstrated that, working in vitro, the chlorophyll content significantly diminished starting from 4 mg L<sup>-1</sup>, with reductions of 31.1 and 83.9% for plants treated with dietholate or not (Karam et al., 2003). The results supported the ones found in this work; however, plants showed a decrease in the chlorophyll content starting from 0.05 mg L<sup>-1</sup>.

When evaluating the initial fluorescence of chlorophyll (Fo) of plants under the effect of clomazone, it was possible to observe, in most species, small alterations with the increase in Fo values to 201.77, 221.55, 265.47 and 299.22 quantum electrons<sup>-1</sup> in the concentration of 0.05 mg L<sup>-1</sup>, in the species *P. oleracea*, *D. lablab*, *S. bicolor* and *S. rhombifolia*. The other species did not suffer significant changes in the Fo value (Figure 4). It was observed that even species with no intoxication symptoms presented small increases in the Fo values, such as *D. lablab*.

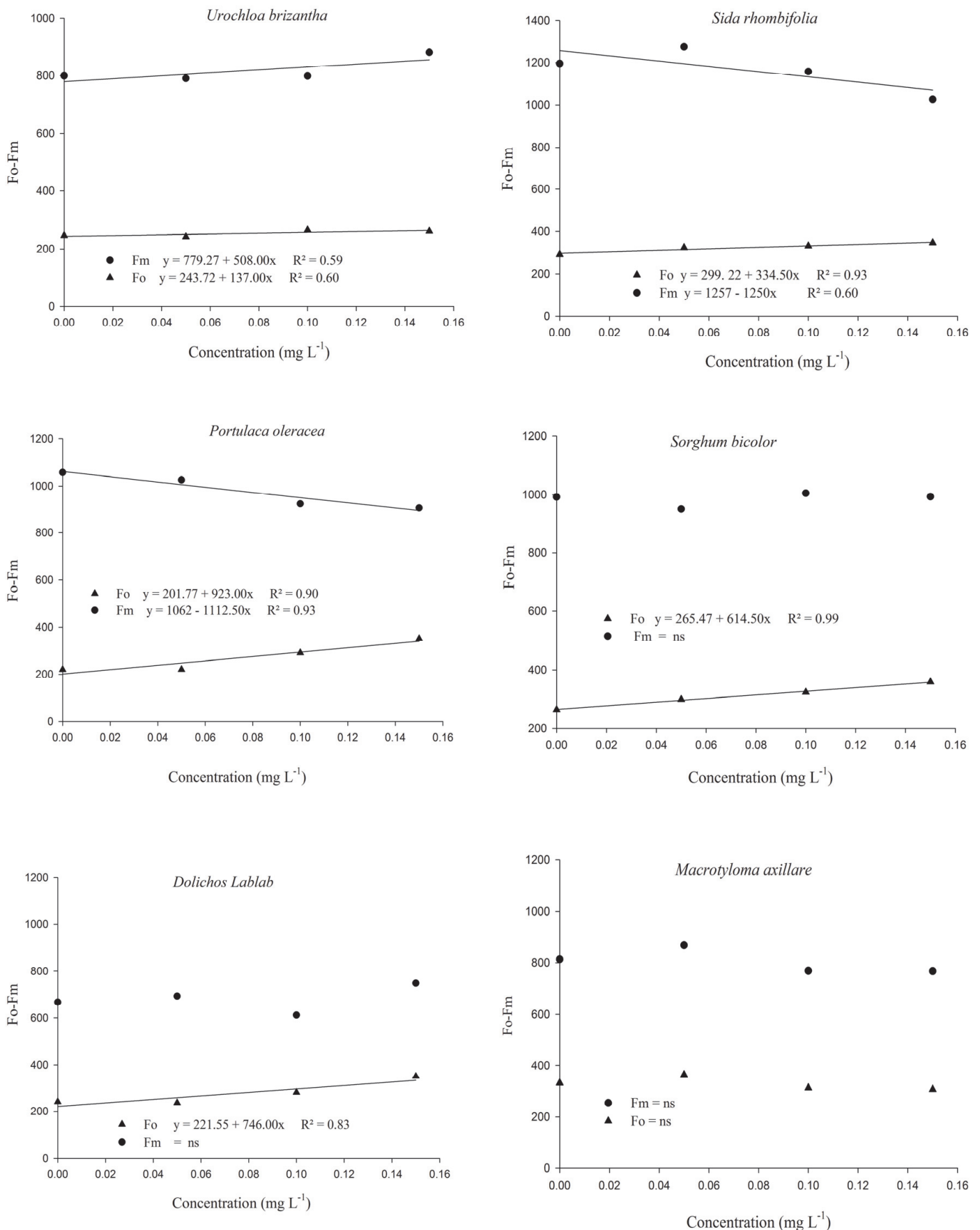
Higher Fo values may indicate structural damages in the reaction centers of the photosystem II or damages in the transportation of excitation energy from the complexes to the reaction centers (Bolhár-Nordenkampf et al., 1989). Fo values may be changed by environmental stresses, which cause structural changes in the photosynthetic pigments of the PSII – in this case, the phyto-toxic effect of clomazone – with the inhibition of the synthesis of carotenoids.

Fo highlights fluorescence when the primary quinone receptor (QA) of the electrons in the photosystem II (PSII) is totally oxidized and the reaction center (P 680) is “active”, indicating the beginning of the photo-chemical reaction (Baker and Rosenqvst, 2004). Thus, the excessive Fo increase reveals destruction of the PSII reaction center or a reduction in the capacity to transfer excitation energy from the antenna to the PSII (Baker and Rosenqvst, 2004).

As for Fm, *U. brizantha* presented a linear increase in Fm values, with a 10% increase under the effect of 0.15 mg L<sup>-1</sup> of clomazone. As for *S. rhombifolia*, the opposite effect was observed, with an average of 910 quantum electrons<sup>-1</sup>, when plants were under the highest herbicide concentration. *P. oleracea* decreased significantly, reducing from 1,062 to 895.125 quantum electrons<sup>-1</sup> at the highest concentration. For this variable, results referring to *S. bicolor*, *D. lablab* and *M. axillare* were not significant; the general average was about 830 quantum electrons<sup>-1</sup>. It is possible to confirm the ineffectiveness of Fm as an effective variable in monitoring the toxic effect of clomazone. Even species with visual intoxication presented normality in this variable.

For what concerns the photo-chemical quenching (Qp), fodder plants and weeds showed different behaviors. *U. brizantha* and *P. oleracea* had their Qp increased by herbicide concentrations, presenting Qp of 0.27 and 0.156 at the 0.05 mg L<sup>-1</sup> concentration and 0.342 and 0.23 quantum electrons<sup>-1</sup> at the last clomazone concentration. The opposite effect was observed for *Dolichos lablab* and *S. bicolor*, where reductions were observed, demonstrating at the last concentration values of 0.184 and 0.23 quantum electrons<sup>-1</sup> (Figure 5). The photo-chemical quenching (Qp) is the energy dissipation caused by the photochemical process, that is, caused by the use of energy to reduce the NAPD within the photosynthetic process. This quenching decreases according to the closing of the reaction centers (QA reduction). When there are significant damages to the structures of photosystems that are directly related to the photo-chemical process, it is possible to notice the reduction of the Qp values coming from damages to the PSII reaction center (Campostrini, 2001).

With the increase in the clomazone concentration and its respective effect over the synthesis of carotenoids and the destruction of chlorophyll, the energy dissipation through the photo-chemical process is reduced, and most of the absorbed energy is dissipated by the non-photochemical quenching (Qn). Some species presenting visual intoxication did not show Qn alterations. *U. brizantha*, *S. bicolor* and *P. oleracea* presented, respectively, averages of 0.191, 0.136 and 0.039 quantum electrons<sup>-1</sup>. As for *M. axillare*, slight increases were observed, with a rate of 0.144 quantum electrons<sup>-1</sup> at the highest concentration. As for *D. lablab* and *S. rhombifolia*, Qn results were not significant, with an average of 0.041 and 0.021 quantum electrons<sup>-1</sup>, respectively (Figure 5). The non-photochemical quenching (Qn) represents all the other energy dissipation ways, mainly heat (Campostrini, 2001). It was expected that, with the increase in the negative effect of clomazone over susceptible species, there would have been a considerable increase in Qn; however, a minimum and linear effect was observed only for *S. bicolor* and



**Figura 4** - Initial fluorescence (Fo) and maximum fluorescence (Fm) of chlorophyll *a* (quantum electrons<sup>-1</sup>) of fodder plants and weeds under the effect of atmospheric clomazone.



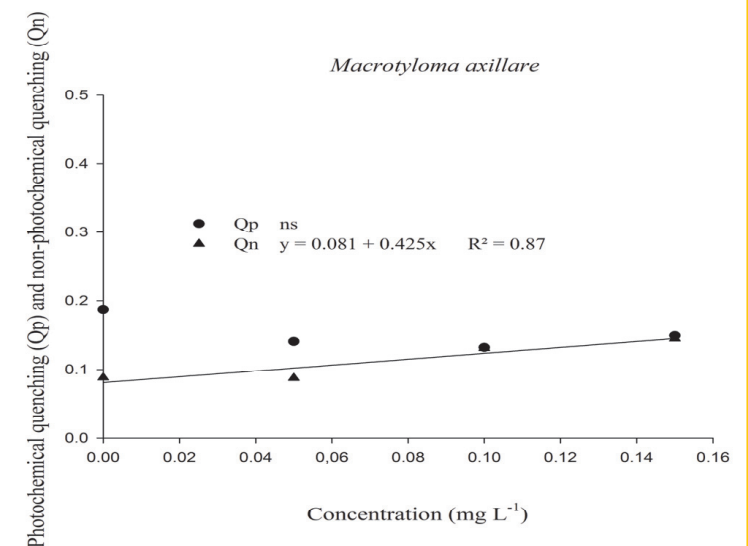
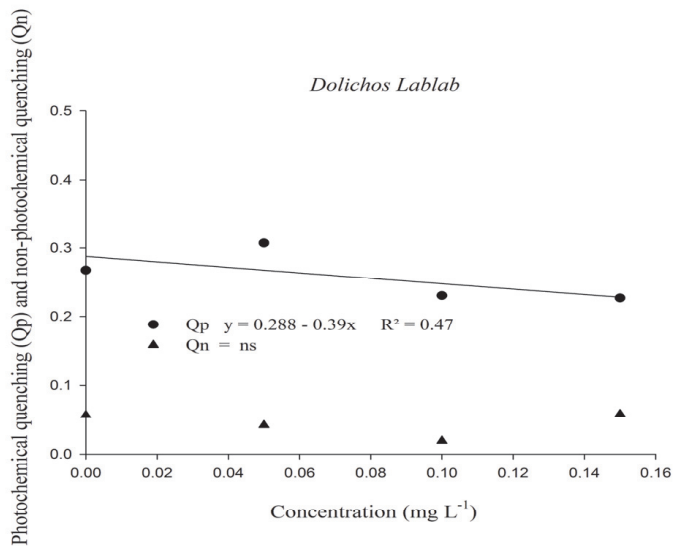
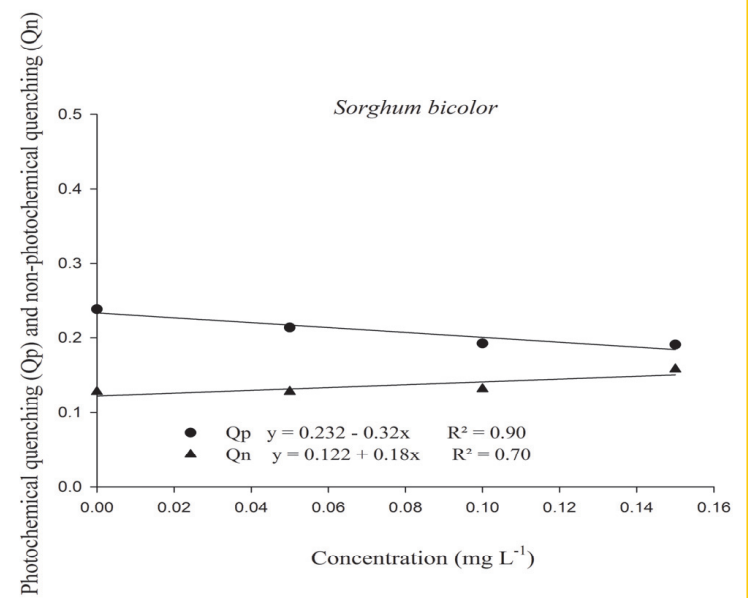
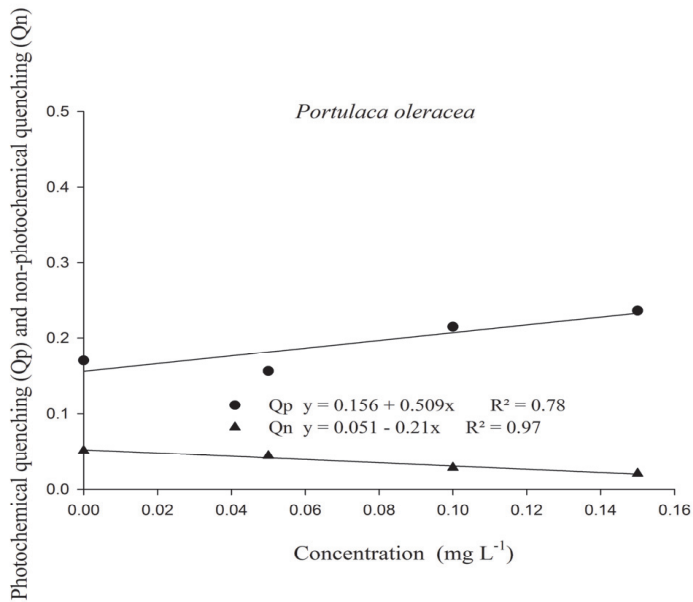
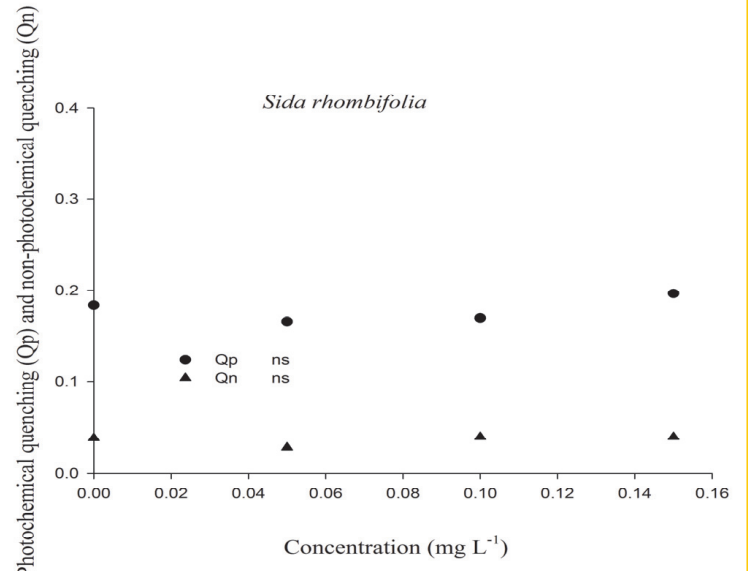
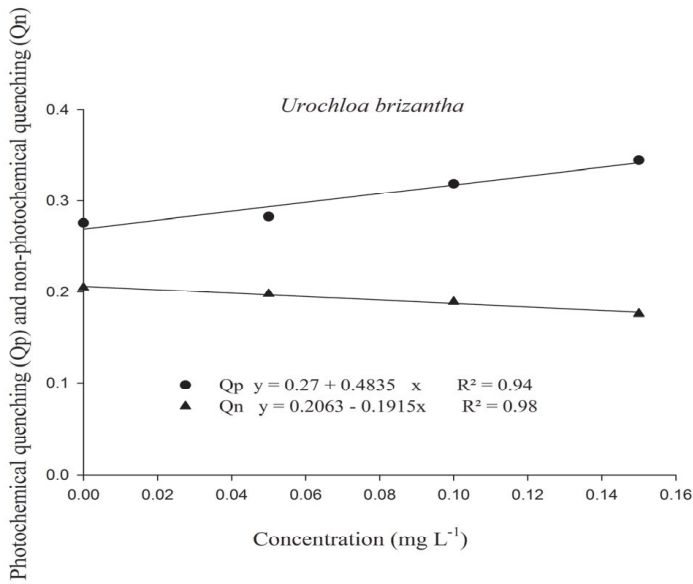


Figura 5 - Photochemical quenching (Qp) and non-photochemical quenching (Qn) of fodder plants and weeds under the effect of atmospheric clomazone.

*M. axillare*. With the destruction of chlorophyll, the energy absorbed by the light is not transported to the photochemical stage, being dissipated through the production of heat in the form of infrared radiation (Campostrini, 2001).

As for the electron transportation rate (ETR), during plant photosynthesis, light is absorbed by chlorophyll and, by exciting electrons, it promotes the transfer of energy to the reaction centers of the photosystems I and II (Young and Frank, 1996). ETR may be negatively altered according to the environmental conditions. Biotic and abiotic stresses may negatively influence the electron transportation rate, mainly due to often irreversible damages in PSII and PSI, impeding the transportation of the electrons that are responsible for the photosynthesis (Bown et al., 2002). However, in this study, ETR was not influenced by the increase in the clomazone concentration; it was one more inviable variable to indicate the presence of the herbicide atmospheric waste (Figure 3B).

In a global analysis of the results, it is worth mentioning that the clomazone concentrations that promoted visual intoxication effects on plants are estimated between 0.05 and 0.15 mg L<sup>-1</sup> and are considered low. There are evidences that an eventual air displacement of clomazone may be dangerous for sensitive cultures, especially fodder and leafy plants.

Clomazone influenced the physiology of all tested species; even in the absence of visual intoxication symptoms, this was perceived at photochemical level, being an important characteristic in the selection of bio-indicators of this molecule in the atmosphere. It is worth mentioning that clomazone is not dangerous for human consumption at the tested concentration, but it may cause damages to the visual aspect of the species, such as leaf albinism, reducing their quality and consumption. For this reason, it is necessary to manage the product properly, avoiding plantations of sensitive species near locations with great clomazone use.

The physiological variables total chlorophyll, maximum quantum yield of the PSII (Fv/Fm) and initial chlorophyll fluorescence (Fo) may be used to monitor effectively clomazone waste in the atmosphere.

According to the results presented when analyzing the physiological variable, it is possible to deduce that *Sorghum bicolor* is the most sensitive species to atmospheric waste of clomazone; it may be a bio-indicator of its presence in the air. Even at concentrations that do not promote visual effects, clomazone may cause significant damages to the photosynthetic activity of the species.

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