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Article

FORAGE PLANTS AND WEEDS THAT ARE SENSITIVE TO ATMOSPHERIC CLOMAZONE RESIDUALS

Plantas Forrageiras e Daninhas Sensíveis a Resíduos Atmosféricos de Clomazone

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ABSTRACT - The use of indicator plants can be an effective alternative in monitoring the presence of toxic molecules in the air, such as herbicides. Thus, in the goal of this study is to assess the sensitivity of forage plants and weeds to atmospheric residual concentrations of clomazone. The treatments were arranged in a 6x5 factorial scheme, with the first factor corresponding to the plant species triticale (*Triticosecale rimpaui*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), palisade grass (*Urochloa brizantha*), purslane (*Portulaca oleracea*) and signal grass (*Urochloa decum*bens), and the second factor to doses of 0, 90, 180, 270 and 360 g ha^{-1} clomazone doses (equivalent to atmospheric concentrations of 0.0, 0.05, 0.10, 0.15 and 0, 20 mg L^{-1}). Twelve days after emergence, the plants were allocated inside experimental rectangular chambers with a volume of 500 dm^3, covered by 150 uM of transparent polyethylene film. The plants remained exclusively in the chamber atmosphere with the herbicide for a period of 96 hours. After the chambers were opened, there were the first evaluation of intoxication and chlorophyll content, as well. The evaluations were repeated on day 7 and 14 after the chamber opening. The presence of clomazone in minimal concentrations in the atmosphere affected the quality of the evaluated plants. Signal grass, palisade grass, sorghum, triticale and purslane were sensitive to atmospheric residual concentrations of clomazone and they can be used to monitor the air quality when there are wastes from this molecule.

Keywords: *Triticosecale rimpaui* Wittm., *Zea mays* L., *Sorghum bicolor* L., *Urochloa brizantha* A. Rich, *Portulaca oleracea* L., *Urochloa decumbens* Stapf.

*RESUMO - O uso de plantas indicadoras pode ser uma alternativa eficiente no monitoramento da presença de moléculas tóxicas no ar, como os herbicidas. Dessa forma, objetivou-se nesta pesquisa avaliar a sensibilidade de plantas forrageiras e daninhas a concentrações residuais atmosféricas de clomazone. Os tratamentos foram arranjados em esquema fatorial 6 x 5, com o primeiro fator correspondendo às espécies vegetais triticale (**Triticosecale rimpaui**), milho (**Zea mays**), sorgo (**Sorghum bicolor**), braquiarão (**Urochloa brizantha**), beldroega (**Portulaca oleracea**) e capim-braquiária (**Urochloa decumbens**), e o segundo, às doses de clomazone de 0, 90, 180, 270 e 360 g ha^{-1} (equivalentes às concentrações atmosféricas de 0,0, 0,05, 0,10, 0,15 e 0,20 mg L^{-1}). Doze dias após emergência, as plantas foram alocadas no interior de câmaras experimentais retangulares, com volume de 500 dm^3, recobertas por filme de polietileno transparente de 150 mm. As plantas ficaram exclusivamente sob a atmosfera da câmara com o herbicida por período de 96 horas. Posteriormente, as câmaras foram abertas e procedeu-se à primeira avaliação da intoxicação e, também, do teor de clorofila. As avaliações foram repetidas aos 7 e 14 dias após abertura da câmara. A presença de clomazone*

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em concentrações mínimas na atmosfera afetou a qualidade das plantas avaliadas. Capim-braquiária, braquiarão, sorgo, triticale e beldroega são espécies sensíveis às concentrações residuais atmosféricas de clomazone, podendo ser utilizadas no monitoramento da qualidade do ar quando há resíduos dessa molécula.

Palavras-chave: *Triticosecale rimpaui* Wittm., *Zea mays* L., *Sorghum bicolor* L., *Urochloa brizantha* A. Rich, *Portulaca oleracea* L., *Urochloa decumbens* Stapf.

INTRODUCTION

Clomazone [2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone] is an herbicide belonging to the chemical group of isoxazolidinones and it is indicated to control, during pre-emergence, monocots and eudicots, mainly in soybean, sugarcane, tobacco, irrigated rice, cotton and cassava cultures (Brazil, 2016).

Currently, clomazone is sold in Brazil in the form of the commercial products Gamit 360 CS®, Gamit 500 EC® and Gamit Star®, whose active ingredient concentration is 360, 500 and 800 grams, respectively (Schreiber et al., 2015). Clomazone is highly water soluble (1,100 mg L^{-1} at 25 °C), with Koc value (organic carbon partition coefficient of soil-water) varying between 150 and 562 cm^3 g^{1}, value of Henry's Law constant 4.19 x 10^{-3} Pa m^3 mol^{-1} and steam pressure of 0.018 Pa (1.35 x 10^{-4} mmHg) at 25 °C. Due to these characteristics of water solubility and steam pressure, clomazone is easily transported by leaching, as well as through water steam which, added with the capacity of volatizing, places clomazone as an important molecule at environmental level (Silva et al., 2012).

In the light of these characteristics of clomazone, the product is easily volatized and, when in the environment, it may cause temporary intoxication on cereals in winter, such as wheat, oat and rye, and also on cultures such as sunflower, medium, horticultural and citrus products. Depending on the concentration, the harmful effect may not be reversible for these cultures. Clomazone is absorbed by the upper organs of plants, such as the leaves; thus, the cultivation of clomazone-sensitive species may be qualitatively affected when they are exposed to herbicide sub-doses (Karam et al., 2003; Rogoli et al., 2008).

An accessible technique to inform about the presence of these toxic pesticides in the environment and a possible problem of ecosystem contamination by herbicide is the use of plants indicating residuals of these products in the soil. This technique is characterized by being low cost. The use of bioindicators is a proper methodology to determine the presence of air pollutants; however, little is known about the indicating capacity of clomazone air residuals (Klumpp et al., 2001; Nunes and Vidal, 2009).

As for the behavior of clomazone in the soil, Inoue et al. (2012), when evaluating the residual effect during pre-emergence on different soils, highlighted that clomazone provided control above 80% over *Urochloa decumbens* plants in soils with clayey texture, with an average dose of 1.0 kg ha^{-1}. On the other hand, Mendes et al. (2012) stated that *U. decumbens* was more sensitive to the presence of clomazone; it was completely controlled with doses above 50% (1.10 kg ha^{-1}) of the recommended one.

Alves et al. (2002), while studying the selectivity of clomazone and other herbicides on *U. decumbens*, *U. brizantha*, *P. maximum* Jacq. cv. Tanzânia and *P. maximum* Jacq. cv. Mombaça, verified that all of them were sensitive to the presence of clomazone. Thus, it is suggested that these species are efficient in monitoring clomazone residuals into the soil. However, it is not known what their behavior is as indicators of air residuals.

Based on the above considerations, the selection of forage species, as well as the selection of weed samples that are commonly found in cultures and for which clomazone is recommended, becomes interesting for bio-indication purposes. Thus, the goal of this work was to evaluate the sensitivity of forage plants and weeds to air residual concentrations of clomazone.

MATERIAL AND METHODS

The experiment was conducted in a monitored environment, with temperature, radiation and luminosity control, belonging to the Faculdade de Ciências Agrárias da Universidade Federal dos Vales do Jequitinhonha e Mucuri, campus JK, Diamantina - Minas Gerais state. It was conducted in chambers inside a greenhouse. The work was set in completely randomized design with five replications, arranged in 6 x 5 factor scheme; in the first factor, these species were allocated: triticale (*Triticosecale rimpaui*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), palisade grass (*Urochloa brizantha*), purslane (*Portulaca oleracea*) and signal grass (*Urochloa decumbens*). In the second factor, clomazone doses were applied: 0, 90, 180, 270 and 360 g ha^{-1} (equivalent to atmospheric concentrations of 0.0; 0.05; 0.10; 0.15; and 0.20 mg L^{-1}). For each clomazone concentration a chamber was used and, inside, six experimental units were placed, which were constituted by 20 x 30 cm plastic trays, 7 cm deep, filled with 3.0 dm^3 of soil.

The soil sample was collected in an area with no herbicide application history, with the predominance of A texture horizon soil, whose chemical analysis presented the following composition: pH water (1:2.5) = 5.74; CTC pH7 = 5.89 $cmol_c$ dm^{-3}; organic matter = 0.80 dag dm^{-3}; Ca = 2.45 $cmol_c$ dm^{-3}; Mg = 0.67 $cmol_c$ dm^{-3}; exchangeable Al = 0.06 $cmol_c$ dm^{-3}; available P = 119.06 mg dm^{-3}; exchangeable K = 337.50 mg dm^{-3}; V = 67%; clay = 6 dag kg^{-1}; sand = 86,20 dag kg^{-1}; and t = 4.05 $cmol_c$ dm^{-3}. The soil was previously sieved and fertilized with 10.0 g of simple superphosphate per tray. The species were planted on lines inside the trays, spaced about 10 cm apart and containing eight seeds per line, from which five plants were later selected through thinning. The experimental spot consisted in chambers with six trays containing the tested species, one tray per species.

Twelve days after emergence, the plants were placed inside the chambers. The chambers were built in a rectangular shape, made of PVC tubes (20 mm) and covered in transparent low density 150 m polyethylene film, µwith the following size: 1.0 x 1.0 m sides, 0.5 m height and 500 dm^3 volume (Figure 1A). The species remained inside the chambers for 96 hours continuously (Figure 1B). Temperatures inside the chambers were monitored during the exposure period of plants to clomazone (Figure 2).

Clomazone was diluted in de-ionized water in the established doses, considering the surface of the growth chamber (1.0 m^2). The solutions were added on Petri dishes, which remained exposed inside the chambers for 96 hours, allowing the evaporation of the product together with the water. There were two dishes per chamber. The plants used as control samples were also placed inside the chambers, but only using de-ionized water. After this interval, the chambers were opened and the trays, together with the dishes, were removed, proceeding to the first evaluation of visual intoxication and chlorophyll, repeated on day 7 and 14 after opening.

The evaluation of plant species intoxication by clomazone was based on the visual scale with 0 to 100% variation, where 0% corresponds to the absence of symptoms and 100% to the total death of the plant (SBCPD, 1995), as well as the chlorophyll content, determined through a CFL1030 digital chlorophyll meter; the results were expressed in µg cm^{-2}.

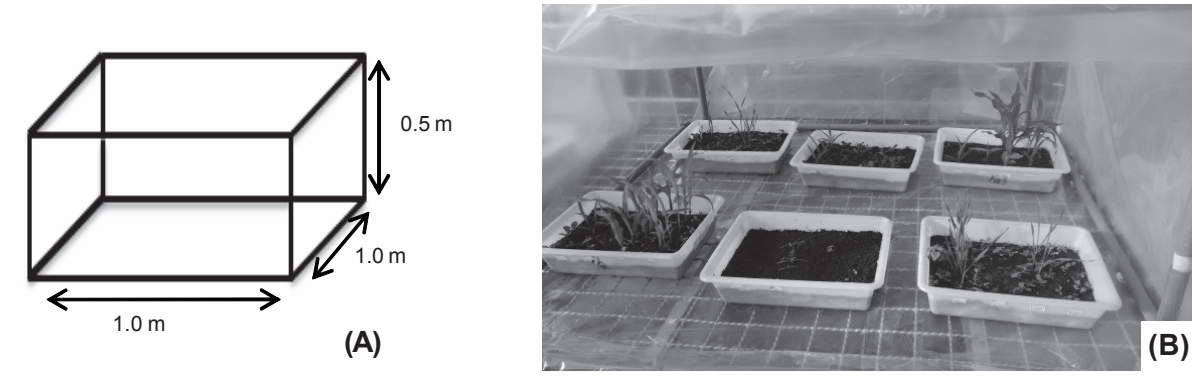

Figure 1 - Representative scheme of the experimental chamber for the exposure of plant species to air residuals of clomazone herbicide.

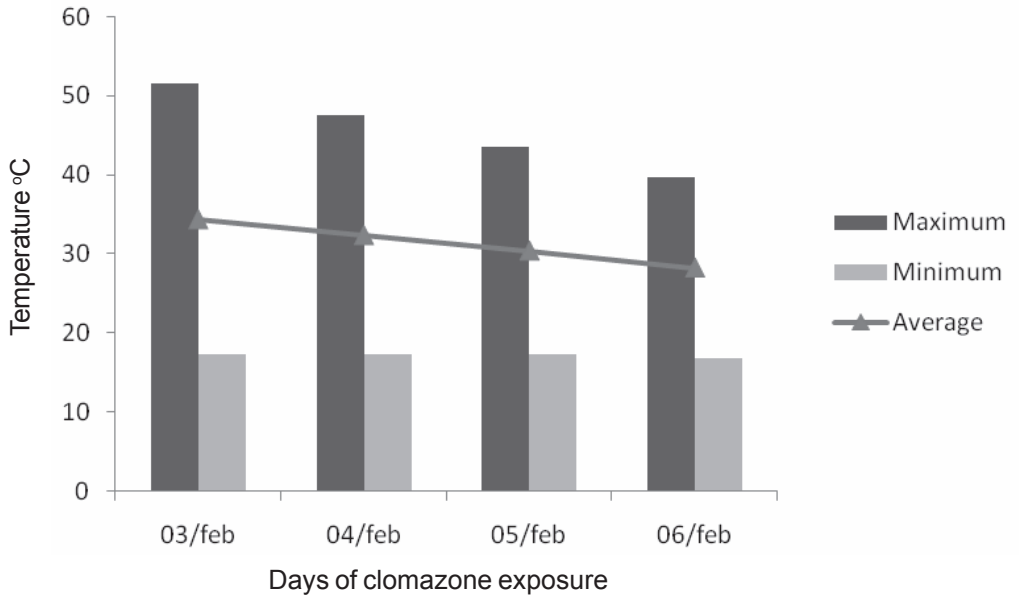

Figure 2 - Maximum, minimum and average temperature inside the experimental chambers during the four days of exposure to clomazone.

Data were submitted to analysis of variance at p>0.05 probability, and the averages of the clomazone concentration effect on each species, when significant, were submitted to regression.

RESULTS AND DISCUSSION

A significant effect was noticed in the tested clomazone concentrations over practically all of the species, with an interaction between the herbicide concentration and the evaluated periods. The increase in the clomazone concentration caused an increase in the intoxication symptoms for all species, in all evaluation periods. Most species presented some potential to indicate the presence of clomazone residuals in the atmosphere, confirmed by intoxication symptoms in the tested species, cause by the clomazone herbicide. Leaves with a whitish color were observed, that is, de-pigmented; this is a characteristic of the clomazone action, responsible of inhibiting the synthesis of carotenoids, which protect chlorophyll (Senseman, 2007).

Clomazone intoxication symptoms on sorghum, triticale, palisade grass, palisade grass and signal grass increased with the evaluation periods. As for triticale, it was observed that the most severe intoxication occurred on the fourteenth day after opening the chamber, reaching 30%. This very same behavior was highlighted also for sorghum and signal-grass. In the case of sorghum, the highest intoxication was found on the fourteenth day, in the concentration of 0.20 mg L^{-1}, with 31% symptoms, whereas for signal-grass, the increase in intoxication symptoms grew only until the seventh day, with average intoxication of 20%. The rise in herbicide concentration caused an increase in the intoxication symptoms until the eighth day for palisade grass and the tenth day for purslane (Figure 3).

Clomazone sensitivity was also related by other authors. Raimondi et al. (2010) verified that, in order to control purslane in pre-emergence in cotton cultures, clomazone provided control above 95% in the lowest dose (125 g ha^{-1}). Cavero et al. (2001) stated that 180 g ha^{-1} doses controlled 100% purslane. These results supported what was found in this research, noticing purslane's susceptibility to clomazone residuals. On the other hand, Mendes et al. (2012), while monitoring the mobility and persistence of herbicides applied into soil, verified that clomazone doses higher than 0.55 kg ha^{-1} were able to control 100% of *U. decumbens* plants, whereas in the same application range, for sorghum plants, there was only 12% control.

Evaluating the intoxication of maize, it was highlighted that it presented higher tolerance to clomazone residuals in the atmosphere; intoxication was not altered with the increase of herbicide

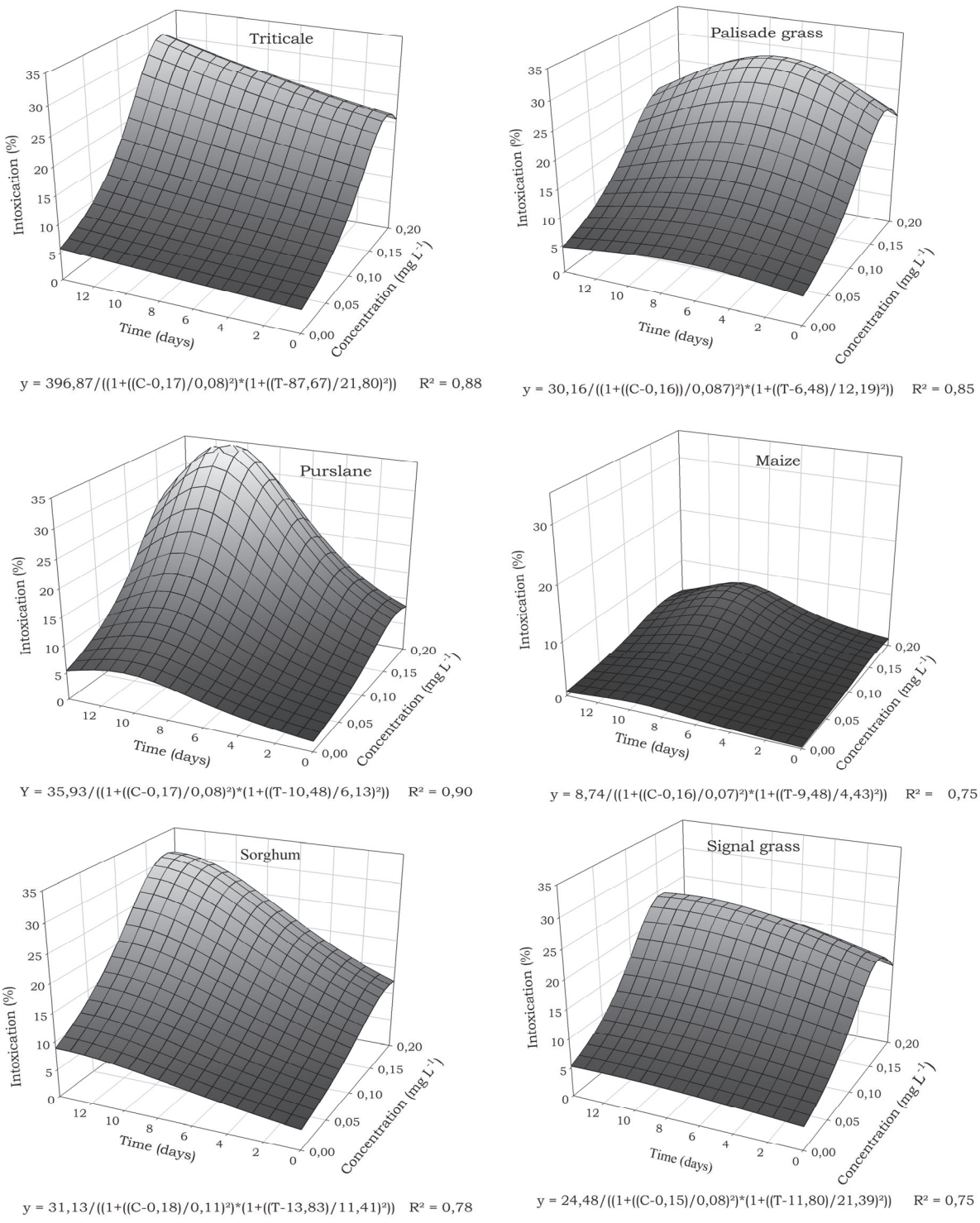

Figure 3 - Estimates of the effects of clomazone concentrations (C) and evaluation periods (T) over intoxication (%) of weed and forage plants.

concentration in no evaluation period. Generally speaking, intoxication did not exceed 10%, demonstrating the species tolerance to the maximum tested herbicide concentration (Figure 3). Similar results were also found by Schreiber et al. (2013), who, while evaluating the susceptibility of plant species to clomazone in the steam phase, observed the gradual increase in the intoxication of sorghum with the time after the exposure, whereas maize presented recovery potential from the symptoms caused by the herbicide starting from the tenth day. These authors also stated that sorghum was the species presenting the highest visual intoxication, followed by

maize and rice. Even admitting intoxication values of 30%, it is possible to observe the sensitivity of the tested species. This differential sensitivity suggests some kind of tolerance for species like maize.

Generally speaking, the differentiated tolerance among plants may be the result of the morpho-physiological and anatomical differences existing among the species. These differences may create obstacles to the input, translocation, time and intensity of exposure of the molecule into the plants, as well as differences in metabolizing the product. These obstacles may justify the differences in the selectivity of clomazone for cultivated species and may be related to morphological and genetic characteristics existing in dicots and monoctos. This may result into different responses of the cytochrome enzyme P450 monooxygenase, giving higher sensitivity to some species and tolerance to others (Deuber, 1992; Schreiber et al., 2013).

The capacity of an herbicide to intoxicate a determined culture is related to a series of factors, among which it is possible to highlight the plant capacity to metabolize the active ingredient of this agrochemical (Cumming et al., 2002). In this case, it is possible to highlight the action of the enzymatic complex cytochrome P-450 and also of the agro-climate conditions at the time of the application, such as high temperatures and low air humidity. High temperatures, as well as facilitating the volatilization of clomazone, may contribute to the phyto-toxic effect of the molecule.

Considering the variation observed in the temperature during the evaluation of the work inside the experimental unit, between 30 and 35 °C (Figure 1), the effect resulting in the microclimate may have contributed for the higher intoxication of these plants even with such reduced product doses. However, it is in this temperature range that the real field effects are expected. Schreiber et al. (2013) state that low temperatures cause lower herbicide activation and, consequently, lower activity of the enzyme cytochrome P450, causing less intoxication. In this work, the observed effect may be opposite, since temperatures close to 33 °C may have increased the action of the enzyme P450, providing higher clomazone activation.

The complexes of P450 enzymes catalyze reactions that may lead to a quick metabolic detoxification from herbicides belonging to the chloroacetanilide, aryloxyphenoxypropionic, sulfonylurea, imidazolinone, isoxazolidinones and sulfonamide groups, protecting the phyto-toxic action of some cultures from the Liliopsida (Mougin et al., 1991; Riechers et al., 2010).

The action of cytochrome P450 occurs in the following way for clomazone: considering clomazone as a pre-herbicide, in order to cause intoxication in the susceptible plant through metabolization, it is necessary that the 2-[(2-chlorophenyl) methyl]-4,4-dimethyl-3-isoxazolidinona)] molecule is transformed into cinco-keto-clomazone, which is the metabolite with herbicide activity (Tenbrook et al., 2006). The molecule transformation into cinco-keto-clomazone possibly occurs by the action of the P450 cytochrome enzyme mono-oxygenase, existing in most plant species. According to Yun at al. (2005), the enzyme is related to the selectivity of certain species to clomazone and various other herbicides, since it is responsible for the metabolization of innumerous molecules. It is believed that the difference in sensitivity of the species tested in this work is related to the presence of the enzymatic complex P450.

Due to the action of clomazone, exclusively inhibiting the synthesis of isoterpernoids, it is likely that, as basic precursors of carotenoids, there is little chlorophyll protection against photo-oxidation. The chlorophyll content of plants was reduced with the increase in the concentration of clomazone residuals in the atmosphere. However, there were differences in the reduction degree for each species. For palisade grass and sorghum, there was a decrease in the chlorophyll content with the increase in clomazone concentration, with a 45% average reduction and content of 33.20 µg cm^{-2} in the control sample treatment, and reaching 17.96 µg cm^{-2} in plants under 0.20 mg L^{-1} concentration. For these two species, the average values found per time were represent, since there was no alteration in the chlorophyll content during the evaluation period (Figure 4).

As for triticale plants, the highest chlorophyll values were also found in the treatment without clomazone and proportional decrease with the concentration increase. However, in relation to the evaluation period, there was no difference between the first day (zero) and the last one (14), which presented averages of 40.75 and 28.85 µg cm^{-2}, respectively. On the seventh evaluation

day, the lowest values of chlorophyll content were found in triticale plants for the concentrations of 32.50 for 14.40 µg cm^{-2}, in the concentration of 0.20 mg L^{-1}. As for purslane, there was no significant difference in the chlorophyll content, even if there was a slight decrease with the increase in clomazone concentration. Likewise, there were no differences between the evaluation periods; values between 29 and 36 µg cm^{-2} were found (Figure 4).

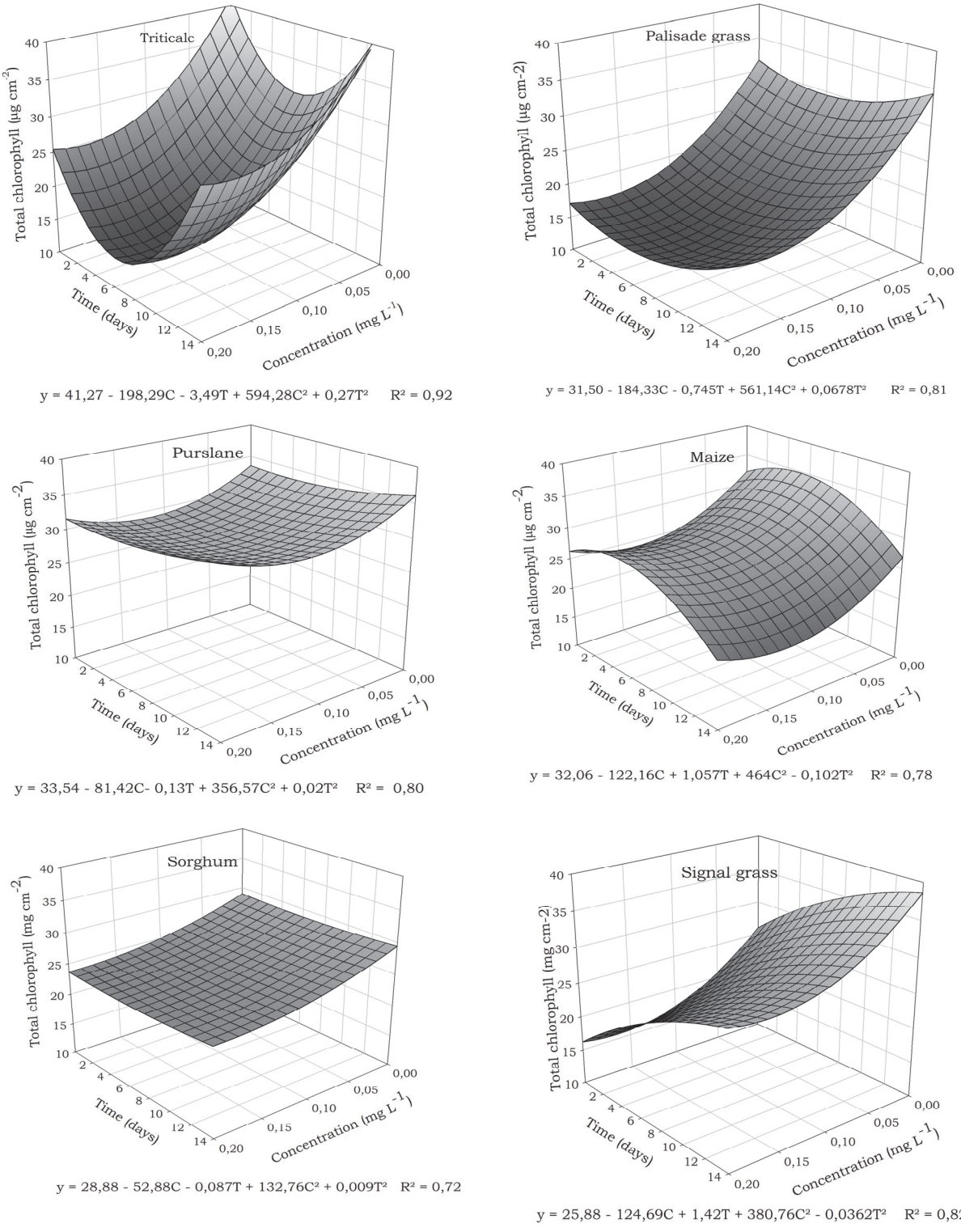

Figure 4 - Estimates of the effects of clomazone concentrations (C) and evaluation periods (T) over the total chlorophyll content (mg cm^{-2}) of weeds and forage plants.

The response of signal grass as for chlorophyll content, increase in clomazone concentration and evaluation period was positive, demonstrating tolerance of the species to the herbicide. During the first evaluation day, as the clomazone concentration increased, the chlorophyll content decreased, reaching the concentration of 0.2 mg L^{-1} with a content that was 53% lower than the initial one (32 µg cm^{-2}). However, with the increase of the evaluation period, the plant managed to recover, presenting chlorophyll content the maximum concentration of 31 µg cm^{-2}, on the fourteenth day (Figure 4).

Under the effect of the clomazone action, the total chlorophyll content is more impaired than the photochemical effectiveness, possibly because the herbicide molecule damages the formation of the chlorophyll pigment and not the transportation of electrons. Kana et al. (2004) studied the photosynthetic capacity of barley (*Hordeum vulgare*) seedlings, cultivated in filter-paper containing 0.25 and 0.5 mM of clomazone, under 12 days of continuous light, and verified reductions in the total content of chlorophyll and also in the levels of carotenoids. They concluded that the photochemical processes in this species cannot fully work, due to the loss of pigments caused by the herbicide intoxication.

The occurrence place of intoxication symptoms caused by clomazone on plants is always close to the meristem of leaves, where the highest synthesis of carotenoids occurs, and also where the greatest quantity of chlorophyll is concentrated. With the continuous exposure of plants to the herbicide (for 96 hours), there were differences in the intoxication of each species. After the exposure, intoxication symptoms were observed and also a reduction of the chlorophyll content. As for maize, the reduction in the chlorophyll content happened after the seventh day, appearing on the fourteenth day with a content between 20 and 25 µg cm^{-2}.

Carotenoids are considered real protectors of chlorophyll; when there is the inhibition of its biosynthesis, the plant is exposed to light excess, which may cause photo-oxidation to chlorophyll, destroying it. The inhibitions of carotenoid synthesis will generate the visual symptom on leaves of sensitive and young plants, which lose their green color and become white; this generates a typical albinism or de-pigmentation symptom (Senseman, 2007; Oliveira Jr, 2011). According to these authors, the reduction in the chlorophyll content is justified, since with the increase of symptoms highlighted by intoxication, the different tested species will remain exposed to the excess of light, and the photo-oxidation will help reducing the total chlorophyll of these species. With the destruction of chlorophyll, the plant diminishes, and in some cases even stops, the photosynthetic activity, which leads the plant to a drastic growth fall and leading to tissue death.

Generally speaking, it is possible to see the potential of clomazone, in the tested concentrations, in causing significant damages to sensitive species, reducing not only the chlorophyll content, but also the plant growth. These concentrations are not enough to cause the senescence of the sensitive plant, even if they may cause significant damages, as the ones observed. It is necessary, however, to emphasize the capacity of this herbicide to reduce the visual quality of the aerial part of innumerous sensitive species, including species that are meant for human consumption. It is necessary to explore more plant species, in order to establish which cultures are more sensitive to the air residual of the herbicide.

The presence of concentrations, even if minimum, in the atmosphere may cause economic damages, which justifies the need for previous knowledge of soil conditions but not only them, but also the air quality, to avoid future damages. Except for maize, the species are interesting as bioindicators, since they are able to present effects even at the estimated clomazone concentration of 0.05 mg L^{-1}.

The presence of clomazone in minimum concentrations in the atmosphere affected the chlorophyll content in the evaluated species, except for purslane. Signal grass, palisade grass, signal grass, triticale and purslane are the most sensitive species to air residual concentrations of clomazone; they can be used to monitor the quality of air.

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