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ELECTROLYTE LEAKAGE AND THE PROTECTIVE EFFECT OF NITRIC OXIDE ON LEAVES OF FLOODED RICE EXPOSED TO HERBICIDES[1]

Extravasamento de Eletrólitos e Efeito Protetor de Óxido Nítrico em Folhas de Arroz Irrigado Expostas a Herbicidas

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ABSTRACT - The nitric oxide acts on the antioxidant system of plants and can discontinue the damage of herbicides elicitors of oxidative stress that cause the disruption of membranes and leakage of cellular contents. In order to evaluate the protective effect of nitric oxide in electrolytes leakage, leaf segments of the Puita INTA CL rice cultivar were incubated with 0, 5, 50, 500 and 5,000 µM clomazone (360 g a.i. L^{-1}), oxadiazon (250 g a.i. L^{-1}), oxyfluorfen (240 g a.i. L^{-1}) and the formulated mixture of paraquat (200 g a.i. L^{-1}) + diuron (100 g a.i. L^{-1}) to obtain the maximum potential conductivity of 50% (MPC$_{50}$). Subsequently, leaf segments were pre-treated with 0, 200 and 2,000 µM of sodium nitroprusside (SNP) for four hours and further incubated for 48 hours with 0, 0.5, 1, 2 and 4 times the concentration of the herbicide that caused the CMP$_{50}$, and the protective effect was reassessed in the presence of nitric oxide scavenger, cPTIO. The MPC$_{50}$ was caused by exposure to 188.9, 273.4, 410.2 + 205.1 and 917.0 µM of Oxadiazon, Oxyfluorfen, Paraquat + Diuron and Clomazone. Pretreatment with 200 µM of SNP reduced electrolyte leakage in leaf segments exposed to 2 and 4 times the MPC$_{50}$ to oxadiazon and paraquat + diuron, while 2,000 µM reduced the damage caused by oxyfluorfen, at the same concentrations. Also, 200 and 2,000 µM of SNP were efficient for clomazone, and the protection was confirmed by cPTIO in all cases.

Keywords: *Oryza sativa*, sodium nitroprusside, clomazone, oxadiazon, oxyfluorfen, paraquat + diuron.

RESUMO - O óxido nítrico atua no sistema antioxidante das plantas e pode conter o dano de herbicidas elicitores de estresse oxidativo, que causam a desorganização de membranas e o extravasamento do conteúdo celular. Com o objetivo de avaliar o efeito protetor do óxido nítrico sobre o extravasamento de eletrólitos, segmentos foliares do cultivar de arroz irrigado Puitá INTA CL foram incubados com 0, 5, 50, 500 e 5.000 µM dos herbicidas clomazone (360 g i.a. L^{-1}), oxadiazon (250 g i.a. L^{-1}), oxyfluorfen (240 g i.a. L^{-1}) e com a mistura formulada de paraquat (200 g i.a. L^{-1}) + diuron (100 g i.a. L^{-1}), para obtenção da condutividade máxima potencial de 50% (CMP$_{50}$). Posteriormente, segmentos foliares foram pré-tratados com 0, 200 e 2.000 µM de nitroprussiato de sódio (SNP) por quatro horas e, após, incubados por 48 horas, com 0, 0,5, 1, 2 e 4 vezes a concentração dos herbicidas que causou a CMP$_{50}$, sendo o efeito protetor reavaliado na presença do sequestrador de óxido nítrico, cPTIO. A CMP$_{50}$ foi causada pela exposição a 188,9; 273,4; 410,2 + 205,1; e 917,0 µM de oxadiazon, oxyfluorfen, paraquat + diuron e clomazone. O pré-tratamento com 200 µM de SNP reduziu o extravasamento de eletrólitos nos segmentos foliares expostos a duas e quatro vezes a CMP$_{50}$ de oxadiazon e paraquat + diuron, enquanto 2,000 µM reduziram o dano causado pelo oxyfluorfen, nas mesmas concentrações, assim como 200 e 2,000 µM de SNP foram eficientes para clomazone, sendo a proteção confirmada pelo cPTIO em todos os casos.

Palavras-chave: *Oryza sativa*, nitroprussiato de sódio, clomazone, oxadiazon, oxyfluorfen, paraquat + diuron.

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INTRODUCTION

The herbicides that inhibit the carotenoid synthesis, protoporphyrinogen oxidase enzyme (protox) and photosystems I and II are widely used in agriculture for the control of weeds. For these herbicides, the exposure to light is crucial to the phytotoxicity, due to generation of reactive oxygen species (ROS), singlet oxygen ($^1O_2^{\bullet}$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) (Foyer et al., 1994) which affects the integrity of the membranes and causes damages to the tissues and, consequently, the death of the plants (Hess, 2000).

The uncontrolled production of ROS resulting from the oxidative stress caused by these herbicides, promotes severe injuries, such as the destruction of chlorophyll, DNA fragmentation, lipid peroxidation and cell content leakage (Dodge, 1994). Nitric oxide (NO), an endogenous free radical or provided through the donor compounds, such as sodium nitroprusside (SNP), has been studied for its ability to contain the damage caused by the oxidative stress in plants (Ferreira et al., 2011), with its protective action confirmed by the use of the nitric oxide sequestrant cPTIO (Neill et al., 2003).

Among the mechanisms that minimize the stress attributed to the nitric oxide are: the induction of defense genes expression (Durner et al., 1998), the activation of superoxide dismutase enzymes (SOD) and ascorbate peroxidase (APX) (Neill et al., 2003), the increase in the content of photosynthetic pigments (Qian et al., 2009), the enabling of internal transportation and maintenance of iron homeostasis, favoring the biosynthesis of chlorophylls and the development of chloroplasts (Graziano and Lamattina, 2005), and the suppression in lipid peroxidation (Belenghi et al., 2007), through reduction in the contents of H_2O_2 and other reactive oxygen species (Hung and Kao, 2003), which results in the preservation of the integrity of the membranes and the reduction of cellular content leakage.

Ion leakage has been used as an efficient parameter for the monitoring of the damage caused by herbicide action mechanisms that affect the integrity of the membranes, whose effects can be rapidly detected by the measurement of the increase in electrolyte conductivity in the solution in which the tissues were submerged (Duke and Kenyon, 1993). Therefore, it is a representation of the injury caused by oxidative stress in the membranes (Falk et al., 2006) and it has been used to assess the potential damage of herbicides such as protox inhibitors (Falk et al., 2006; Trezzi et al., 2011, Glomski; Netherland, 2013), photosynthetic electron transport inhibitors and carotenoid synthesis inhibitors (Kruse et al., 2006). In the crop of flooded rice, these herbicides come as an alternative for weed resistance management, and their selectivity, conditioned by several physiological and environmental factors, would be benefitted by the use of protectors.

This paper aimed at assessing, *in vitro*, the protective effect of nitric oxide, provided through SNP, on electrolyte leakage of leaf segments from flooded cultivar Puitá Inta CL, exposed to different concentrations of herbicides oxadiazon, oxyfluorfen, clomazone and to the formulated mixture of paraquat + diuron.

MATERIAL AND METHODS

The Puitá INTA CL cultivar was grown in an appropriate area for the production of flooded rice, in the city of Formigueiro (latitude of $30^{o}3'59''$S and longitude of $53^{o}33'7''$W), Rio Grande do Sul (RS), Brazil. The sowing density used was 350 seeds m^{-2} and the management practices adopted were the ones recommended by technical recommendations (Sosbai, 2012), but without the application of phytosanitary products. At the V_3 development stage (Counce et al., 2000), the plants were transferred with soil to vases and taken to the laboratory, where three complementary experiments were conducted for each one of the herbicides. Therefore, rice leaves without hems were washed in distilled water and dried in paper towel for the collection of 0.5 g of leaf segments of approximately 0.25 cm^2, which were put in tubes to centrifuge containing 30 mL of incubation solution, according to the recommendations of each trial described below.

To determine the dosage that allowed the maximum potential conductivity of 50% (MPC_{50}), the leaf segments were incubated in solutions (pH 6.5) containing 1.0 mM of the MES buffer (2-(N-morpholino) ethanesulfonic acid), 1% ($p\ v^{-1}$) of saccharose and the herbicide. That way, concentrated solutions were prepared of the commercial herbicides clomazone (360 g a.i. L^{-1}), oxadiazon (250 g a.i. L^{-1}) and oxyfluorfen (240 g a.i. L^{-1}) and of the formulated mixture of paraquat (200 g a.i. L^{-1}) and diuron (100 g a.i. L^{-1}) and dilutions were done in order to obtain concentrations of 5, 50, 500 and 5,000 µM of active ingredient. In the formulated mixture, the concentrations obtained for diuron were 2.5, 25, 250 and 2,500 µM. In the control, the segments were incubated only with the buffer solution. The use of MPC_{50} enables the evaluation of the herbicide action, minimizing the effect of the solution concentration in the ion leakage.

The solution's electrolyte conductivity was measured (conductivimeter Tec-4MP TECNAL, adjusted to 25 °C) at 5, 24 and 48 horas after the beginning of incubation, which happened in a growth chamber at a temperature of 25 ± 2 °C and constant fluorescent light, under 2,500 lux (digital lux meter LD-200 INSTRUTHERM), and these conditions were used for all the experiments. After the last reading, the tubes were incubated for two hours in water bath, at 75 ± 2 °C, to obtain maximum conductivity – procedure adopted in all the experiments. The conductivity data was transformed into maximum conductivity percentage (Falk et al., 2006), and they were expressed in maximum potential conductivity (%) (Dayan et al., 1997). The concentrations and the incubation periods, in a factorial design, consisted of treatments organized under an entirely randomized design with four replications.

After defining the MPC_{50}, leaf segments were incubated for four hours in solutions of 200 and 2,000 µM of SNP, followed by the control (distilled water). After this period, the solution was completely removed and replaced by the incubation medium previously described, in the concentrations of zero (only incubation solution), 0.5, 1, 2 and 4 times the concentration of the herbicide corresponding to MPC_{50} (Table 1). The conductivity measurement of the solution was done 48 hours after the beginning of incubation. Later on, the samples were heated in water bath to obtain maximum potential conductivity. The data was expressed in MPC (%), and the SNP concentration levels, combined with the concentration of the herbicides, consisted of treatments, organized in an entirely randomized design with four replicates.

In order to validate the stress mitigating factor imposed by the nitric oxide, 0.2 g of leaf segments were incubated in 12 mL of distilled water (control), SNP solution or SNP solution followed by 200 µM of cPTIO for the period of four hours, in the previously described conditions. Then, the solutions were completely removed and the incubation medium was added, containing the buffer solution and the herbicide in the previously tested concentrations of 378.0, 546.8, 1,835.9 and 820.3 + 410.2 µM, respectively, for oxadiazon, oxyfluorfen, clomazone and paraquat + diuron. The electrolyte conductivity of the solution was measured 48 hours after the beginning of incubation with the herbicides and after cooling the tubes, in order to obtain maximum leakage of electrolyte.

All data obtained was assessed as to their normality (Shapiro-Wilk) and variance homogeneity (Levene) and transformed ($arcsen(Vx/100$), whenever necessary, to serve

Table 1 - Concentrations of the herbicides, proportional to 0.5, 1, 2 and 4 times the necessary concentration to cause 50% of maximum potential conductivity (MPC_{50}), in leaf segments of Puitá INTA CL flooded rice cultivar

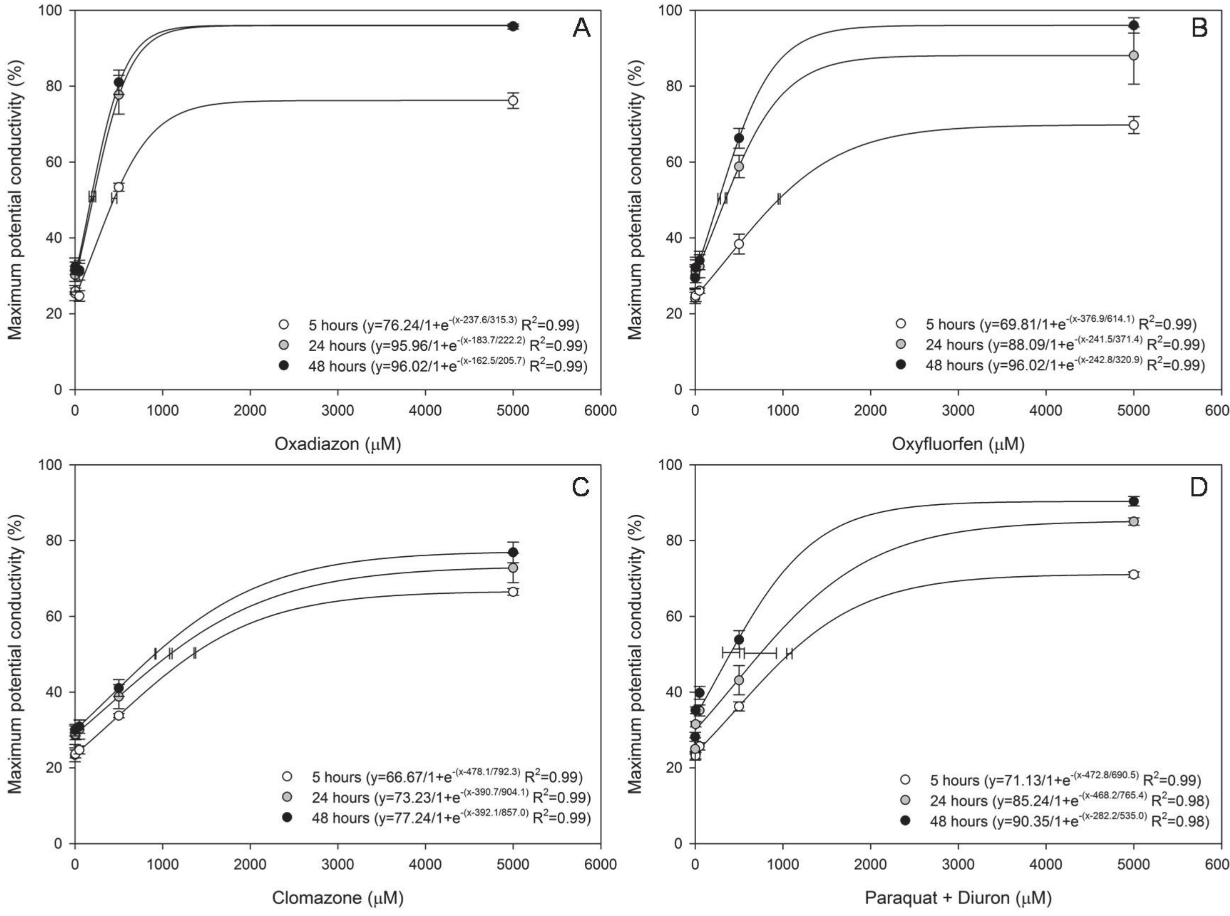
Herbicide	0.5	1	2	4
	Concentration (µM of a.i.)			
Oxadiazon	94.5	188.9	378.0	756.0
Oxyfluorfen	136.7	273.4	546.8	1093.6
Paraquat + Diuron	205.1 + 102.9	410.2 + 205.1	820.3 + 410.2	1,640.6 + 820.3
Clomazone	458.9	917.9	1,835.9	3,681.9

the assumptions of the model. The data from experiments 1 and 2 was subjected to a variance analysis, with later regression, while the data from experiment 3 was subjected to the t test ($p>0.05$), with a pairwise comparison of treatments SNP or SNP + cPTIO with herbicides without pre-treatment.

RESULTS AND DISCUSSION

There was interaction between the concentrations of oxadiazon and oxyfluorfen herbicides and the formulated mixture of paraquat and diuron and the incubation periods (Figure 1A, B and D), while for clomazone the interaction was not statistically significant (Figure 1C). However, since the determination of the concentrations that caused the maximum potential conductivity of 50% (MPC_{50}) enables the comparison among the herbicides, the results obtained were adjusted by regression in all cases.

The percentage of electrolytcs released in the incubation solution, resulting from the damage to the membranes, increased progressively with the increase in the concentration of the herbicides; the same happened for the increasing incubation periods. Data adjustment by the equations enabled the calculation of the MPC_{50}. After 48 hours of incubation, the necessary amount was 188.9, 273.4, 410.2 + 205.1, and 917.0 µM



Vertical bars correspond to the standard deviation of the means, while the horizontal bars correspond to the confidence interval in 95% error probability of the dosage that causes 50% of electrolyte leakage (MPC_{50}). Santa Maria, 2015.

Figure 1 - Maximum potential conductivity (%) of the leaf segments of the flooded rice cultivar Puitá INTA CL exposed to different concentrations of oxadiazon (A), oxyfluorfen (B), clomazone (C) and paraquat + diuron (D) incubated for 5, 24 and 48 hours.

of oxadiazon, oxyfluorfen, paraquat + diuron and clomazone, respectively, so that such percentage was reached, the same order found for the incubation periods of 5 and 24 hours. Nevertheless, there was no significant difference between the incubation periods of 24 and 48 hours for the oxadiazon herbicide, so, in this case, the lowest concentration was adopted, relative to the incubation period of 48 hours, as a reference for the following experiment (Figure 1A).

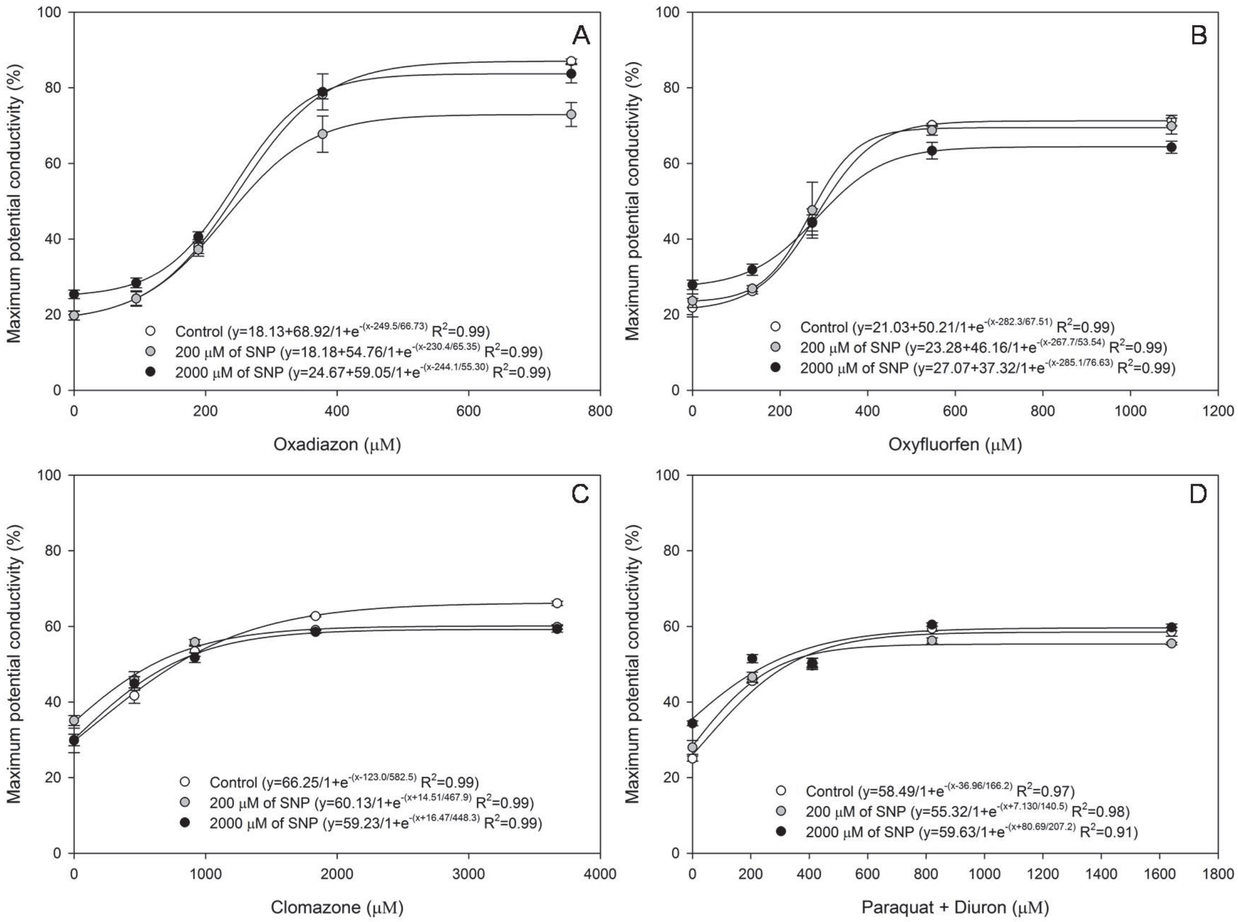
Paraquat, a photosystem I inhibitor herbicide, just like the protox inhibitors, oxadiazon and oxyfluorfen, acts fast on the plants. All of them need light to act, but photosynthesis is a requirement for the activity of the herbicides that act in the photosynthetic transport of electrons, which is the case of paraquat and diuron (Duke et al., 1991). In addition, the authors also highlight the ability the protox inhibitors have of causing accumulation of protoporphyrin IX, a photodynamic compound that, in the cytoplasm and in the presence of light, converts rapidly the molecular oxygen into singlet oxygen, even with inhibition of a small amount of the chloroplast enzyme.

The pre-herbicide clomazone needs to be converted in the active metabolite 5-cetoclomazone (Dayan and Watson, 2011) to then inhibit 1-deoxy-D-xylulose-5-phosphate synthase (Ferhatoglu and Barrett, 2006), at methylerithritol phosphate pathway (MEP) of production of isoprenoids, synthesized in chloroplasts (Eisenreich et al., 2004), resulting in the reduction of carotene and phytol levels and, consequently, chlorophyll levels (Timossi and Alves, 2001). Since the carotenoids also have a protecting role in the photosystems, by dissipating the excess of chlorophyll energy in the excited state, the herbicide action needs to equally have a flow of electrons in the photosystems. These considerations may explain the greater activity of oxadiazon and oxyfluorfen, in smaller concentrations, when compared to the formulated mixture of paraquat + diuron and the clomazone herbicide.

When exposed to pre-treatment with SNP and, later on, to different concentrations of herbicides, the behavior of the electrolytic conductivity in the solution depended on the combination of the levels of both factors, indicating significant interaction between them for all assessed herbicides. Pre-treatment of the leaf segments with 200 μM of SNP reduced ion leakage in the concentrations of two and four times the MPC_{50} of oxadiazon (Figure 2A) and paraquat + diuron (Figure 2D), while the concentration of 2,000 μM of SNP caused reduction, in the same concentrations as oxyfluorfen (Figure 2B). Both concentrations of SNP reduced the release of electrolytes of the segments treated with two and four times the MPC_{50} of clomazone (Figure 2C), and there was no significant difference between 200 and 2,000 μM of SNP, in this case.

Although the leaf segments that were pre-treated with SNP and exposed to the concentrations of herbicides of two to four times the MPC_{50} have presented reduction of 3.1 to 14.1% in electrolytes leakage, such as in the case of paraquat + diuron and oxadiazon, respectively, these concentrations of SNP provided a small increase in the maximum potential conductivity, when isolated or in smaller concentration of herbicides. The effects of nitric oxide in plants have been long associated to their concentration (Anderson and Mansfield, 1979), being considered an inducing agent (Leshem, 1996) or stress mitigator (Hsu and Kao, 2004).

The ambiguous behavior of nitric oxide, acting as a powerful oxidizing agent and antioxidant effect, has also been reported by Popova and Tuan (2010), pointing out the concentration and the characteristics of the species, the environment and the eliciting stress as conditions. According to these authors, under severe stress conditions and consequently ROS, the NO can interrupt the lipid peroxidation chain, limiting the damage, while in isolation it can act as a reactive nitrogen species and start chain reactions, causing injuries to the cell. According to Hayat et al. (2010), the cytoprotective role of nitric oxide is based on its ability to keep redox homeostasis and regulate the toxicity level of reactive oxygen species. Caro and Puntarulo (1998) point out the modulation in the formation of superoxide and inhibition of lipid peroxidation as protection mechanisms.



Vertical bars correspond to the standard deviation of the means. Santa Maria, 2015.

Figure 2 - Maximum potential conductivity (%) of the leaf segments of the Puitá INTA CL flooded rice cultivar exposed to pre-treatment with 0, 200 and 2,000 μM of sodium nitroprusside and incubated for 48 hours with different concentrations of oxadiazon (A), oxyfluorfen (B), clomazone (C) and paraquat + diuron (D).

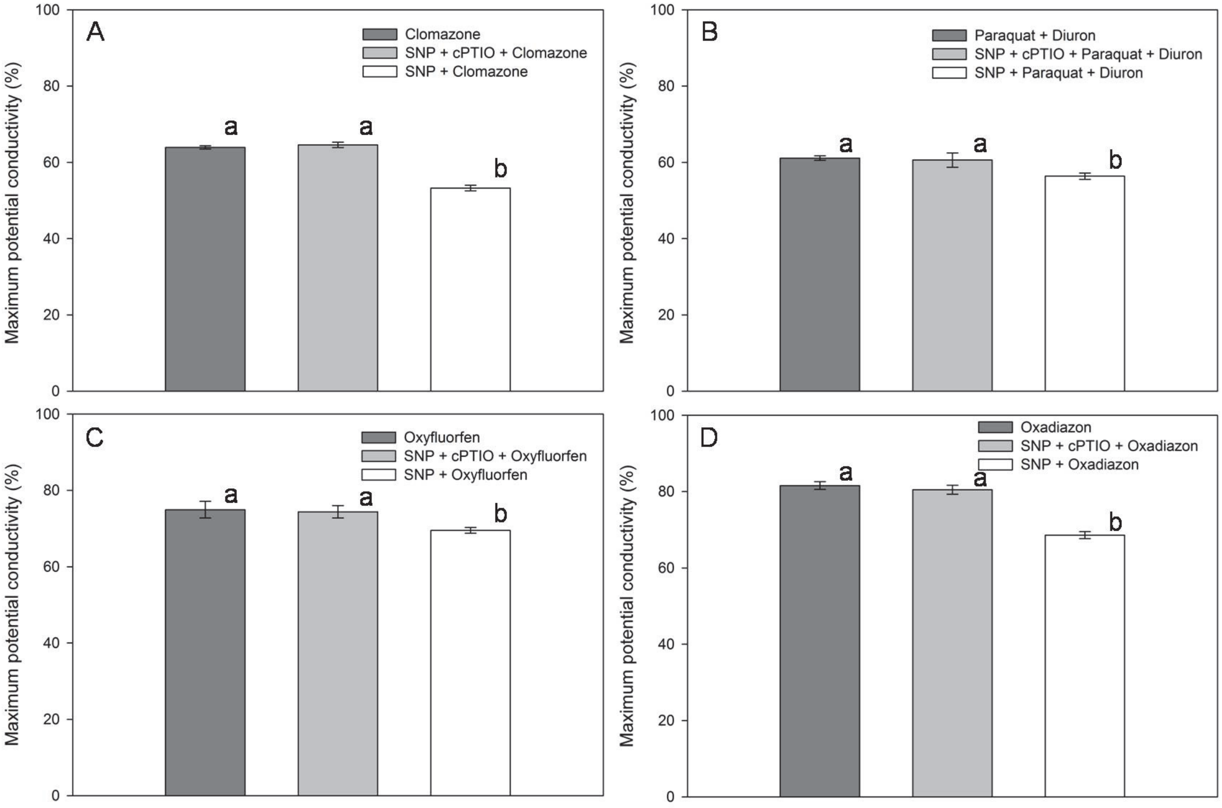
In biologic systems, the nitric oxide radical ($NO^•$) reacts really fast with superoxide, producing peroxynitrite ($ONOO^-$), and the formation rate depends on the concentration of the parent molecules. In physiologic pH, peroxynitrite is protonated, forming peroxynitrous acid (ONOOH), which, depending on its concentration, is quickly decomposed to nitrate (NO_3^-) or hydroxyl radical (Popova and Tuan, 2010). Although it also has a cytotoxic action, peroxynitrite and its byproducts might act as powerful modulators of redox regulation in several signal transduction pathways (Liaudet et al., 2009), through nitration of proteins (Arasimowicz-Jelonek and Floryszak-Wieczorek, 2011). However, the exact mechanism that determines the toxic or cytoprotective action of $ONOO^-$, endogenous or produced by NO reaction provided exogenously, with the superoxide resulting from oxidative stress, is still unknown (Arasimowicz-Jelonek and Floryszak-Wieczorek, 2011).

The 2-4-carboxyphenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide (cPTIO) is used as nitric oxide scavenger (D'Alessandro et al., 2013), in order to prove its effect in biological systems. The maximum potential conductivity obtained in treatments with 200 μM of SNP + 378.0 μM of oxadiazon, 2,000 μM of SNP + 546.8 μM of oxyfluorfen, 2,000 μM of SNP + 1,835.9 μM of clomazone and 200 μM of SNP + 820.3 μM of paraquat and

410.2 µM of diuron, followed or not by cPTIO, is shown on Figure 3.

Due to their capacity of reacting with NO in the medium, the pre-incubated treatments with SNP + cPTIO present maximum potential conductivity statistically similar to the injury caused by the isolated herbicide, confirming the NO protective effect in the reduction of ion leakage, in several levels, for all the tested herbicides. Although they have well characterized the action conditioned by light, the destruction of polyunsaturated fatty acids of the membranes and the consequent necrotic tissue, these herbicides have different eliciting factors from the oxidative process. Oxadiazon and oxyfluorfen cause the formation of singlet oxygen in cytosol, while clomazone, by absence of dissipation of the excess of chlorophyll energy, causes the formation of triplet chlorophyll and singlet oxygen in the chloroplasts, just like diuron. Paraquat, when interacting with FS I, promotes the formation of superoxide (Hess, 2000), which can explain the smaller protection percentage between the assessed action mechanisms, considering the possibility of a fast reaction between NO and o $O_2^{\bullet -}$, forming $ONOO^-$. Moreover, the synergism between the action mechanisms of paraquat and of diuron (Costa et al., 2013) potentially promotes greater amount of stress causing agents, which, in the presence of 2,000 µM of SNP, can produce harmful levels of $ONOO^-$, explaining the toxicity of the higher concentration of the protector in these conditions.

The *in vitro* treatment of rice leaf segments with SNP reduced the electrolyte leakage, resulting from damage to the membranes



Means followed by equal letters do not differ from each other by the t test (p>0.05), while the vertical bars represent the means standard deviation. Santa Maria, 2015.

Figure 3 - Maximum potential conductivity (%) of the leaf segments of the Puitá INTA CL flooded rice cultivar exposed to pre-treatment with distilled water, sodium nitroprusside and/or cPTIO and incubated for 48 hours with 1,835.9; 820.0+410.2; 546.8; e 378.0 µM, respectively, of (A) clomazone, (B) paraquat + diuron, (C) oxyfluorfen and (D) oxadiazon.

caused by herbicides oxadiazon, oxyfluorfen, clomazone and paraquat + diuron, which indicates the protective effect of nitric oxide on the oxidative stress produced by such elicitors; however, in the absence of stress, high concentrations of SNP may cause the loss of cellular ions.

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REFERENCES

Anderson L., Mansfield TA. The effects of nitric oxide pollution on the growth of tomato. **Environ Poll.** 1979;20:113-21.

Arasimowicz-Jelonek M., Floryszak-Wieczorek J. Understanding the fate of peroxynitrite in plant cells from physiology to pathophysiology. **Phytochemistry**. 2011;72:681-8.

Belenghi B. et al. Metacaspase activity of Arabidopsis thaliana is regulated by S-nitrosylation of a critical cysteine residue. **J Biol Chem.** 2007;282:1352-8.

Caro A., Puntarulo S. Nitric oxide generation by soybean embryonic axes. Possible effect on mitochondrial function. **Free Rad Resp.** 1999;31:205-12.

Costa NV. et al. Directed-spray application of paraquat and diuron in physic nut plants. **Planta Daninha**. 2013;31:987-96.

Counce P., Keisling T.C., Mitchell J.A. A uniform, objective, and adaptive system for expressing rice development. **Crop Sci.** 2000;40:436-43.

D'Alessandro S. et al. Limits in the use of cPTIO as nitric oxide scavenger and EPR probe in plant cells and seedlings. **Front Plant Sci.** 2013;4:1-7.

Dayan F.E., Watson S.B. Plant cell membrane as a marker for light dependent and light-independent herbicide mechanisms of action. **Pest Biochem Physiol.** 2011;101:182-90.

Dayan F.E. et al. Soybean (*Glycine max*) cultivar differences in response to sulfentrazone. **Weed Sci.** 1997;45:634-41.

Dodge A. Herbicide action and effects on detoxification processes. In: Foyer C.H., Mulineaux P.M. **Causes of potoactive stress and amelioration of defense systems in plants**. Boca Raton: CRC, 1994. p.219-36.

Duke S.O., Kenyon W.H. Peroxidizing activity determined by cellular leakage. In: Boger P., Sandman G. **Target assays for modern herbicides and related phytotoxic compounds**. Boca Raton: Lewis, 1993. p.61-6.

Duke S.O. et al. Protoporphyrinogen oxidase-inhibiting herbicides. **Weed Sci.** 1991:39:465-73.

Durner J., Wendehenne D., Klessig D.F. Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP ribose. **Proc Nat Acad Sci USA**. 1998;95:10328-33.

Eisenreich W. et al. Biosynthesis of isoprenoids via the non-mevalonate pathway, **Cell Molec Life Sci.** 2004;61:1401-26.

Falk J.S., Al-Khatib K., Peterson D.E. Rapid assay evaluation of plant response to protoporphyrinogen oxidase (Protox)-Inhibiting Herbicides. **Weed Technol.** 2006;20:104-12.

Ferhatoglu Y., Barrett M. Studies of clomazone mode of action. **Pest Biochem Physiol.** 2006;85:7-14.

Ferreira L.C. et al. Morphological and physiological alterations induced by lactofen in soybean leaves are reduced with nitric oxide. **Planta Daninha**. 2011;29:837-47.

Foyer C.H., Leiandais M., Kunert K.J. Photooxidative stress in plants. **Physiol Plant.** 1994;92:696-717.

Glomski L.M., Netherland M.D. Use of a small-scale primary screening method to predict effects of flumioxazin and carfentrazone-ethyl on native and invasive, submersed plants. **J Aquatic Plant Manage.** 2013;51:45-8.

Graziano M., Lamattina L. Nitric oxide and iron in plants: an emerging and converging story. **Trends Plant Sci.** 2005;10:4-8.

Hayat S. et al. A. Nitric oxide: Chemistry, biosynthesis, and physiological role. In: Hayat S. et al. **Nitric oxide in plant physiology**. Weinheim: John Wiley & Sons, 2010. p.1-16.

Hess F.D. Review Light-dependent herbicides: An overview. **Weed Sci.** 2000;48:160-70.

Hsu Y.T., Kao C.H. Cadmium toxicity is reduced by nitric oxide in rice leaves. **Plant Growth Regul.** 2004;42:227-38.

Hung K.T., Kao C.H. Nitric oxide counteracts the senescence of rice leaves induced by hydrogen peroxide. **J Plant Physiol.** 2003;160:871-9.

Kruse N.D. et al. Estresse oxidativo em girassol (*Helianthus annuus*) indica sinergismo para a mistura dos herbicidas metribuzin e clomazone. **Planta Daninha**. 2006;24:379-90.

Leshem Y.Y. Nitric oxide in biological systems. **Plant Growth Regul.** 1996;18:155-69.

Liaudet L., Vassalli G., Pacher P. Role of peroxynitrite in the redox regulation of cell signal transduction pathways. **Frontiers Biosci.** 2009;14:4809-14.

Neill S.J., Desikan R., Hancock J.T. Nitric oxide signalling in plants. **New Phytol.** 2003;159:11-35.

Popova L., Tuan T. Nitric oxide in plants: properties, biosynthesis and physiological functions. **Iranian J Sci Technol Trans A**. 2010;34:173-83.

Qian H. et al. The effect of exogenous nitric oxide on alleviating herbicide damage in *Chlorella vulgaris*. **Aquatic Toxicol.** 2009;92:250-7.

Reunião Técnica da Cultura do Arroz Irrigado, 29ª. 2012: Gravataí, RS. Arroz irrigado: Recomendações técnicas da pesquisa para o Sul do Brasil. Itajaí: Sociedade Sul-Brasileira de Arroz Irrigado - SOSBAI, 2012. 188p.

Timossi P.C., Alves P.L.C.A. Efeitos da deriva de clomazone, aplicado isoladamente ou em mistura com ametryn, sobre características produtivas de laranjeira hamlin. **Planta Daninha**. 2001;19:295-304.

Trezzi M.M. et al. Eletrolite leakage as a technique to diagnose *Euphorbia heterophylla* biotypes resistant to ppo-inhibitors herbicides. **Planta Daninha**. 2011;29:655-62.