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Article

Selectivity of Imazapic + Imazapyr Herbicides on Irrigated Rice as Affected by Seed Treatment with Dietholate and Clomazone Applied in Preemergence

Seletividade do Herbicida Imazapyr + Imazapic no Arroz Irrigado em Função do Tratamento de Sementes com Dietholate e a Aplicação de Clomazone em Pré-Emergência

PIVETA, L.B.[1]
PINTO, J.J.O.[1]
AVILA, L.A.[1]*
NOLDIN, J.A.[2]
SANTOS, L.O.[1]

ABSTRACT - The use of dietholate seed treatment, as well as the use of preemergence clomazone, can affect the selectivity of the imidazolinones chemical group of postemergence herbicides applied in crops Clearfield™. The aim was to evaluate the morphophysiological effects of a formulated mixture of imazapyr + imazapic applied in postemergence in combination with seed treatment with dietholate and applying in preemergence of clomazone on Avaxi CL rice hybrid cultivar. The experiment was conducted in the agricultural year 2012/13, in a greenhouse. The experimental design was completely randomized in a factorial design with four replications, in which factor A consisted of seed treatment with dietholate and applied clomazone herbicide in preemergence on rice and factor B consisted of eight doses of the mixture of imazapyr + imazapic herbicides. The results showed that the rice seed treatment with dietholate combined with the spray of herbicide clomazone in preemergence affects the selectivity of postemergence formulated mixture of imazapic + imazapyr applied on Avaxi CL rice. Physiological characteristics such as photosynthetic rate, transpiration rate and stomatal conductance on Avaxi CL hybrid are negatively affected.

Keywords: *Oryza sativa*, selectivity, photosynthesis, chemical control.

RESUMO - O uso de dietholate no tratamento de sementes, bem como a utilização de clomazone em pré-emergência, pode afetar a seletividade de herbicidas do grupo químico das imidazolinonas aplicados em pós-emergência em cultivares Clearfield®. O objetivo deste trabalho foi avaliar os efeitos morfofisiológicos da mistura formulada do herbicida imazapyr + imazapic aplicada em pós-emergência em combinação com o tratamento de sementes com dietholate e a aplicação em pré-emergência de clomazone no cultivar híbrido de arroz Avaxi CL. O experimento foi conduzido no ano agrícola de 2012/13, em casa de vegetação. O delineamento experimental utilizado foi inteiramente casualizado, em esquema fatorial, com quatro repetições, no qual o fator A foi composto pelo tratamento de sementes com dietholate e aplicação em pré-emergência do arroz do herbicida clomazone, e o fator B, por oito doses da mistura formulada dos herbicidas imazapyr + imazapic. Os resultados mostraram que o tratamento de sementes de arroz com dietholate combinado com a aplicação de clomazone em pré-emergência afeta a seletividade do herbicida composto pela mistura formulada de imazapyr + imazapic aplicada em pós-emergência no híbrido de arroz Avaxi CL. Características fisiológicas, como taxa fotossintética, taxa de transpiração e condutância estomática, no híbrido Avaxi CL são afetadas negativamente.

Palavras-chave: *Oryza sativa*, seletividade, fotossíntese, controle químico.

*** Corresponding author:**
<laavilabr@gmail.com>

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[1] Universidade Federal de Pelotas, Pelotas-RS, Brasil; [2] Epagri – Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Itajaí-SC, Brasil.

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INTRODUCTION

Rice (*Oryza sativa*) is one of the most produced and consumed cereals worldwide. Among the factors that limit the productive potential of irrigated rice crops, weed competition stands out. The use of the Clearfield® technology and seed treatment with dietholate aiming to increase the selectivity of the herbicide clomazone to rice are viable and efficient tools to control several weeds.

The herbicide clomazone is used in preemergence or initial postemergence of the crop to aid in the control of weeds as it has a residual effect and good control spectrum (Andres et al., 2013). However, the use of dietholate with clomazone in conjunction with other herbicides in the postemergence period may cause selectivity problems since dietholate inhibits the action of some enzymes of the Citocromo P450 family, which is one of the means the crop uses to detoxify the herbicides (Sanchotene et al., 2010; Martini et al., 2015).

The use of herbicides alone or in combination can cause injury to the rice crop and consequently reduce its productivity. The extent of such damages may vary depending on the product used, dose applied, cultivar tolerance, environmental factors and other aspects related to the technology adopted by the producer.

Thus, herbicides phytotoxic effects on the crop should not be determined solely by appearance since examples of products that may reduce crop productivity without causing visually detectable effects are already known. However, other herbicides can cause severe injuries, which disappear with the development of the crop without compromising productivity (Camargo et al., 2012).

Therefore, it is necessary to evaluate plants physiological parameters because there are several factors that directly or indirectly influence photosynthesis. Water deficit, thermal stress, internal and external concentrations of gases and light composition and intensity are some of the main parameters (Concenço et al., 2008), besides the limitation of CO_2 concentration at the carboxylation sites in chloroplasts on leaves (Xiong et al., 2015).

Among the physiological variables that can be analyzed, perspiration rate, photosynthetic rate and stomatal conductance stand out. Perspiration in most plant species, including irrigated rice, is determined by climatic demand related to solar radiation, physiological mechanisms related to stomatal responses to environmental factors, leaf area index and soil water availability (Taiz and Zieger, 2013). Likewise, stomatal conductance can be understood as one of the physiological mechanisms that vascular terrestrial plants have to control perspiration, together with mesophyll conductance (Lauteri et al., 2014).

In this sense, seed treatment with dietholate may negatively interfere with the selectivity of the formulation of herbicides imazapyr + imazapic for rice in the Clearfield® technology. Therefore, the objective of this study was to evaluate the morphophysiological effects of the formulation of herbicides imazapyr + imazapic applied in postemergence in conjunction with the seed treatment with dietholate and the application in preemergence of clomazone in Avaxi CL rice hybrid cultivar.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse in the 2012/13 growing season using the Avaxi CL hybrid rice seeds. The experimental units consisted of polyethylene pots with a capacity of 1.5 L. The pots were filled with soil sieved and without clods classified as solodic eutrophic Hydromorphic Planosol with the following characteristics: $pH_{water}(1:1) = 5.1$; CTC pH 7 = 5.4 $cmol_c\ dm^{-3}$; organic matter = 1.2%; clay = 15%; texture = 4; Ca = 1.8 $cmol_c\ dm^{-3}$; Mg = 1 $cmol_c\ dm^{-3}$; Al exchangeable = 0.2 $cmol_c\ dm^{-3}$; P available = 4.3 mg dm^{-3}; K exchangeable = 30 mg dm^{-3}. When the experiment was being carried out, the average temperature was 28 °C. Fertilization, irrigation management and other crop practices were carried out according to the recommendations for irrigated rice cultivation (SOSBAI, 2014).

The experimental design was completely randomized in a factorial arrangement with four replications. Factor A consisted of four tillages of seed treatment (ST) with dietholate and the application in preemergence of rice of clomazone herbicide (720 g a.i. ha^{-1}). Tillage M1 did not receive seed treatment nor the application of clomazone. Tillage M2 received seed treatment but clomazone was not applied in preemergence. Tillage M3 received, besides the seed treatment with dietholate, the application of clomazone on the microencapsulated (CS) formulation. Finally, in the fourth tillage (M4), seeds were treated with dietholate and received the application of clomazone in the emulsifiable concentrate (EC) formulation. Factor B consisted of eight doses of the formulation of herbicides imazapyr + imazapic: at 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, and 2.5 times the commercial dose of herbicide Kifix® (140 g p.c. ha^{-1}) and a control without application of the herbicide (dose zero).

The different doses of the formulation imazapyr + imazapic were applied when the plants reached stage V3 (SOSBAI, 2014). After the application of the herbicide in postemergence the plants were kept in flooding with a water depth measuring approximately 7 cm.

The variables determined were: phytotoxicity, plants height, shoots dry matter (SDM) and variables related to photosynthesis. Phytotoxicity evaluations were done visually, assigning ranking grades of 0 (without symptoms) to 100% (dead plants) at 14, 21 and 28 days after the application of the treatments (DAT). Plants height, i.e., the distance from the plant base between the soil surface row to the youngest leaf apex, was measured at 21, 28 and 35 DAT with the aid of a millimeter calibrated ruler.

The variables photosynthetic rate (A), perspiration rate (E) and stomatal conductance (gs) related to photosynthesis were determined at 14 DAT by an infrared gas analyzer (IRGA, model LI 6400 XT).

At 35 DAT, the plants were cut close to the ground and transfered to a forced air circulation oven at a temperature of 60 ± 5 °C until reaching constant weight. Subsequently, the shoots dry matter mass was determined in an analytical balance.

The effects of seed treatment management with dietholate were analyzed by the Tukey's test ($p \leq 0.05$) and dose effects by the quadratic polynomial regression model ($p \leq 0.05$). The choice of models was based on statistical significance (F test) in the adjustment of the coefficient of determination (R^2) and in the model biological meaning, $y = a + bx + cx^2$, where a: estimated maximum value for the response variable; b: slope of the curve; and x: dose of the herbicide imazapyr + imazapic.

To determine the confidence interval of each point of the curve, the following equation was used: CI = (t x stdev)/root no., where CI = confidence interval; t = tabulated t value, at 5% probability; stdev = standard deviation; and root no. = square root of the number of repetitions.

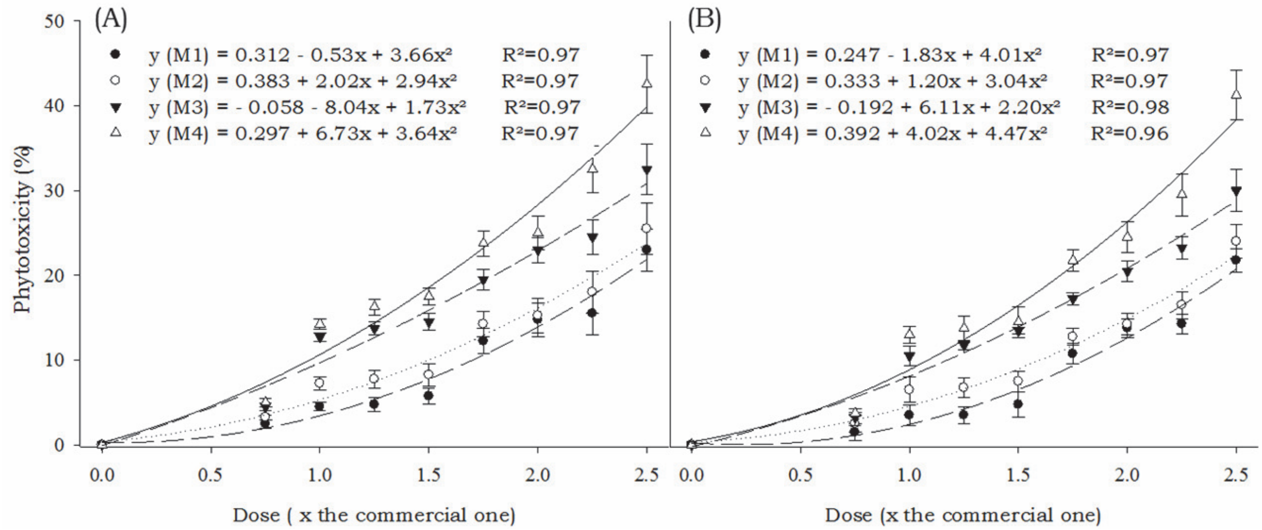
RESULTS AND DISCUSSION

The first variable evaluated in carrying out the experiment was the phytotoxic effect of herbicides on rice plants, which could be visually identified through characteristic symptoms attributed to each of the chemical groups used.

The analysis of variance showed a significant interaction between the tillage factor and the herbicide dose for the variable phytotoxicity evaluated at 14 and 21 DAT (Figure 1). In this first evaluation, the phytotoxicity levels observed increased with the dose and also with the tillage adopted, being M1 < M2 < M3 < M4.

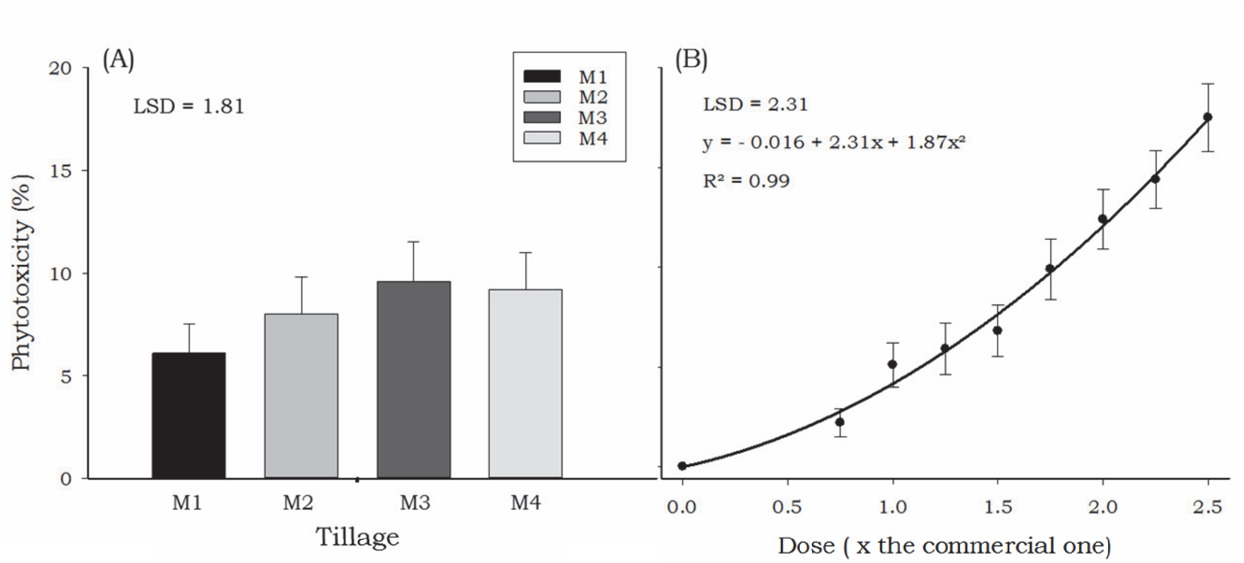
Evaluation held at 21 DAT (Figure 1B) showed behavior that was similar to the previous one's. The were no significant variations in the phytotoxicity levels from 14 to 21 DAT. However, there was a tendency to decrease symptoms at all doses and tillages. In parallel to the dose increase, in general it was observed that the greatest injuries observed in rice were higher in tillages M3 and M4, comparing to tillages M1 and M2. The results indicate that seed treatment only with dietholate and without the application of clomazone in preemergence did not interfere with the selectivity of the herbicide imazapyr + imazapic applied in postemergence.

In the evaluation at 28 DAT (Figure 2) there was no interaction among factors. However, there were significant differences for seed treatment tillages and for herbicide doses. Results found in Figure 2A demonstrate that the phytotoxicity was kept high in tillages M4 and M3, with



The vertical bars represent the confidence interval calculated for each point of the curve.

Figure 1 - Average phytotoxicity (%) at 14 (A) and 21 (B) DAT evaluated in the hybrid Avaxi CL due to doses of the formulation of imazapyr + imazapic applied in postemergence of the rice plants, in plants from seeds pre-treated with dietholate and with the application of clomazone in preemergence, forming the following treatments: M1 – there was no ST and no application of clomazone; M2 – there was ST and no application of clomazone; M3 – there was ST and application of clomazone with a microencapsulated formulation – CS (C); and M4 – there was ST and application of clomazone with emulsifiable concentrate formulation – EC.



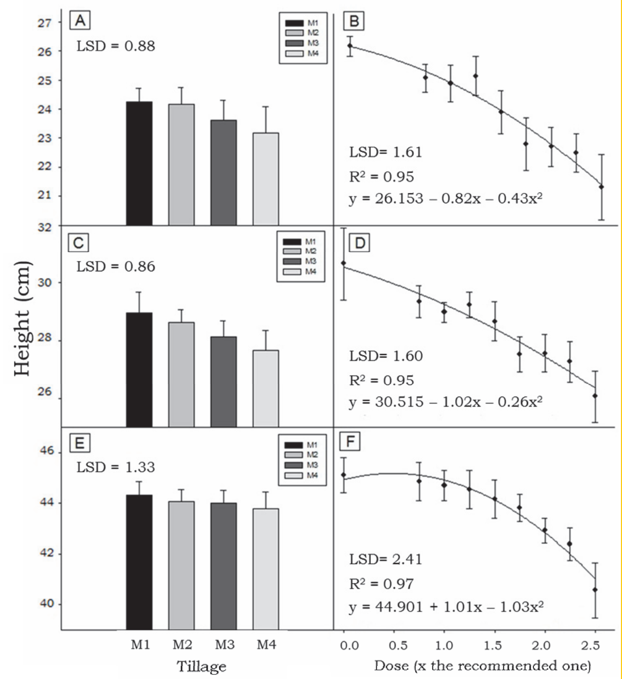
The vertical bars represent the confidence interval calculated for each point of the curve.

Figure 2 - Average phytotoxicity between seed treatment tillage (A) and herbicides doses (B) to rice plants at 28 DAT evaluated in the hybrid Avaxi CL due to the application of the herbicide treatments of the formulation of imazapyr + imazapic. M1 – there was no ST and no application of clomazone; M2 – there was ST and no application of clomazone; M3 – there was ST and application of clomazone with a microencapsulated formulation – CS (C); and M4 – there was ST and application of clomazone with emulsifiable concentrate formulation – EC. LSD: least significant difference.

average values of 9.6% and 9.2%, respectively. However, the values observed from 21 to 28 DAT show increasing recovery of the plants affected. In this evaluation, the results make evident that the injuries still persisting were due mainly to the increase in the herbicide dose (Figure 2B). In general, it was observed that the herbicide phytotoxicity levels to the rice plants due to dose and tillage decreased from the first one (14 DAT – Figure 1) to the last evaluation (28 DAT – Figure 2).

The herbicides formulation from the chemical group of imidazolinones initially caused chlorosis of young leaves with subsequent evolution to necrosis of these leaves. Symptoms similar to those in herbicides in the chemical group of imidazolinones in this and other crops were also observed by authors who have previously studied these herbicides (Tan et al., 2006; Shaner, 2014). Concerning the symptoms attributed to the herbicide clomazone, bleaching of the leaves was observed, followed by necrosis. These symptoms are characteristic of the phytotoxicity presented by herbicides inhibiting the synthesis of carotenes and when the damage is severe they can cause plant death (Scherder et al., 2004; Shaner, 2014).

For the evaluations of rice plants height (21, 28 and 35 DAT), there was no interaction among the factors. However, there was a difference among tillages at 21 and 28 DAT (Figures 3A and C) and among doses of herbicide imazapyr + imazapic at 21, 28 and 35 DAT (Figures 3B, D and F). The plants average height observed in tillages M3 and M4 up to 28 DAT (Figures 3A and C) was lower comparing to tillages M1 and M2. However, when the evaluation was carried out at 35 DAT (Figure 3E), there were no differences in plants height among treatments, indicating that the plants recovered from the injuries caused by the herbicide clomazone. The curve that represents the results showed that there is a significant reduction in the plants average height with the increase of dose of herbicide imazapyr + imazapic.



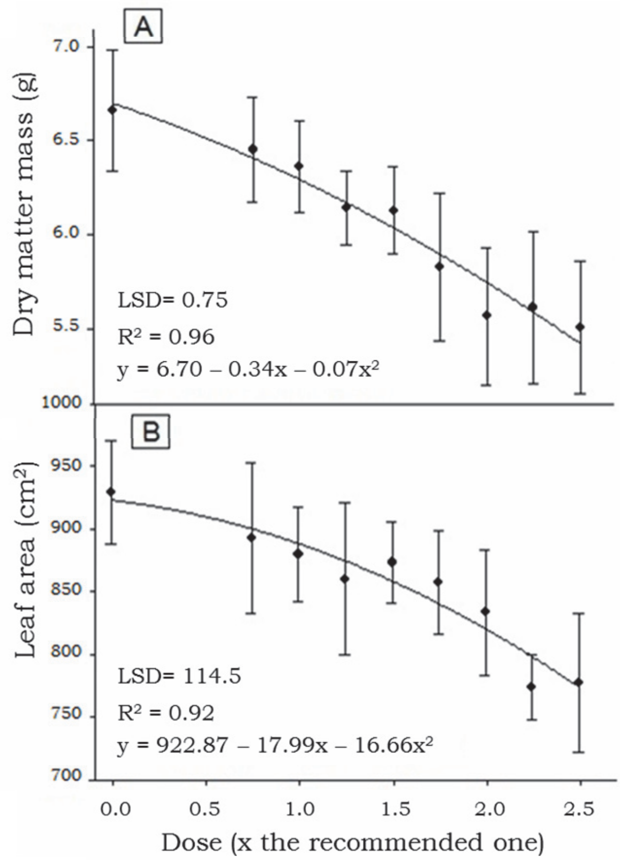
The vertical bars represent the confidence interval calculated for each point of the curve.

Figure 3 - Average height (cm) of irrigated rice plants at 21 (A and B), 28 (C and D) and 35 (E and F) DAT evaluated in hybrid Avaxi CL due to the application of the herbicide treatments of the formulation of imazapyr + imazapic and the seed treatment with dietholate. M1 – there was no ST and no application of clomazone; M2 – there was ST and no application of clomazone; M3 – there was ST and application of clomazone with a microencapsulated formulation – CS (C); and M4 – there was ST and application of clomazone with emulsifiable concentrate formulation – EC. LSD: least significant difference.

The shoots dry matter (SDM) variable answer (Figure 4) shows results with a tendency that is similar to the plants height. Data analysis showed a significant difference for doses of the herbicide imazapyr + imazapic. The results showed that there was a reduction in the SDM production of 4.7% for once the dose recommended and of 19.6% for twice as much the dose, respectively.

As for the variables related to photosynthesis (Figure 5), such as photosynthetic rate (A), perspiration rate (E) and stomatal conductance (gs), there was no interaction among the factors, only differences among tillages and the herbicide doses. Regarding the photosynthetic rate (A), it was possible to see reduction of the parameters, being M1 > M2 > M3 > M4 with values between 26.2 and 21.9 µmol CO_2 m^{-2} s^{-1} for tillages M1 and M4, respectively (Figure 5A). When this variable is analyzed according to the herbicide doses (Figure 5B), it is possible to see a decrease in the photosynthetic rate with increasing herbicide doses. According to Concenço et al. (2008), the photosynthetic rate is directly related with the consumption of CO_2 of the environment and with increasing plants mass. With increasing herbicide dose, the rice plants phytotoxicity level increased (Figures 1 and 2), causing paralysis in the growth and foliar necrosis, which possibly determined the reduction in the floor foliar area for the accomplishment of the photosynthesis.

Photosynthesis and hence respiration depend on constant inflow and outflow of CO_2 and O_2 in the cell. Such free flow is due to the CO_2 and O_2 concentration in the intercellular spaces,



The vertical bars represent the confidence interval calculated for each point of the curve.

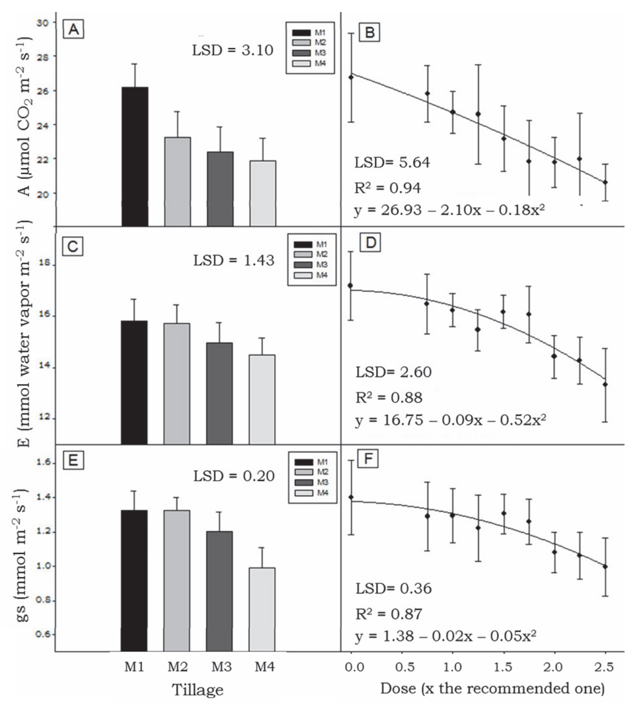
Figure 4 - Shoots dry matter (SDM) and leaf area of irrigated rice plants at 35 DAT evaluated in the hybrid Avaxi CL due to the application of the herbicide treatments of the formulation of imazapyr + imazapic and the seed treatment with dietholate. LSD: least significant difference.

which depend on the stomatal opening, the main control mechanism of the gases movement (Lauteri et al., 2014). This important cellular structure is in turn largely controlled by the swelling of both the guard cells and the epidermal cells of the stomata (Ashraf and Harris, 2013).

The perspiration rate (E) had a behavior similar to that of the photosynthetic rate (A) for the evaluation of the tillages. Tillages M1 and M4 had the values with higher amplitude, which ranged between 15.8 and 14.5 mmol water vapor m^{-2} s^{-1}, respectively (Figure 5C). Concerning the herbicide doses evaluation (Figure 5D), the perspiration rate decreased with increasing dose, ranging from 16.9 to 13.1 mmol water vapor m^{-2} s^{-1} from the control without herbicide application to the highest dose (2.5 x the registration dose), respectively.

The decline in perspiration is associated with the closure of stomata and variations in stomatal opening, which cause changes in water potential as they act on perspiration (Andrés et al., 2014). The plant, therefore, tends to close the stomata when light levels are below photosynthetically active radiation or to avoid water stress (Merilo et al., 2014).

High rates of perspiration by the plant may be beneficial to its development as this factor is directly correlated with the photosynthetic rate. The reduction of leaf perspiration reinforces the idea of the stress promoted by the action of the herbicide of the chemical group of imidazolinones, which can cause metabolic imbalance and collapse and disarrangement in the formation and maintenance of plant structures, due to the inhibition of the synthesis of branched-chain amino acids (BCAA) valine, leucine and isoleucine (Roman et al., 2007).



The vertical bars represent the confidence interval calculated for each point of the curve.

Figure 5 - Photosynthetic rate (A and B), perspiration (C and D) and stomatal conductance (E and F) evaluated in the hybrid Avaxi CL at 14 DAT due to the herbicide application of the formulation of imazapyr + imazapic and the seed treatment with dietholate. M1 – there was no ST and no application of clomazone; M2 – there was ST and no application of clomazone; M3 – there was ST and application of clomazone with a microencapsulated formulation – CS (C); and M4 – there was ST and application of clomazone with emulsifiable concentrate formulation – EC. LSD: least significant difference.

Stomatal conductance (gs) showed the same behavior as in the perspiration rate (E) and the photosynthetic rate (A). Tillages M1 and M4 had the most different values, which ranged between 1.33 and 0.99 mol m^{-2} s^{-1}, respectively (Figure 5E). Concerning the herbicide doses evaluation (Figure 5F), gs decreased with increasing dose, ranging from 1.40 to 1.00 mol m^{-2} s^{-1} from the control without herbicide application to the highest dose (2.5 x the registration dose), respectively.

According to Brodribb and Holbrook (2003), stomatal conductance is proportional to the number and size of stomata and the diameter of the stoma opening, characteristics that depend on other endogenous and environmental factors. However, under stress conditions, the plant tends to close its stomata as a defense mechanism against loss of water, increasing resistance and, consequently, reducing stomatal conductance (Taiz and Zeiger, 2013).

The stomatal closure influences the reduction of perspiration. Stomatal conductance is responsible for the inflow and outflow of water and CO_2 by the stomata. The smaller its opening, the greater the stomatal resistance and consequent decrease in perspiration (Taiz and Zeiger, 2013). The presence of herbicides reduces stomatal conductance in sensitive plants and often in tolerant plants. This usually occurs by the closure of the stomata, which is influenced by several biotic and abiotic factors (Lawlor and Tezara, 2009) such as the use of herbicides (Galon et al., 2010).

The imazapyr + imazapic formulation action on photosynthesis is indirect, because its action is on the synthesis of branched-chain amino acids (BCAA) valine, leucine and isoleucine, responsible for the synthesis of some essential proteins in plants. As for clomazone, it is an inhibitor of the synthesis of carotenoids, compounds responsible for the protection of chlorophyll against excess light (Silva et al., 2007). In addition, chlorophyll can suffer damage from reactive oxygen species (ROS) (Barbosa et al., 2014). As oxidative stress increases, there is a cascade reaction with increased lipid peroxidation, membrane damage, protein damage, especially ATP synthase and vacuolar ATPase (V-ATPase), nucleic acids and thylakoids, which lose their ability to perform photosynthesis (Neilson et al., 2010). The application of herbicides leads to changes in photosynthesis and the increase of ROS in peanut, arabidopsis, rice and wheat plants (Agostinetto et al., 2016; Nohatto et al., 2016; Radwan and Fayez, 2016; Sun et al., 2016).

The treatment of rice seeds with dietholate combined with the application of clomazone in preemergence affects the selectivity of the herbicide composed by the formulation of imazapyr + imazapic applied in postemergence in the Avaxi CL rice hybrid. Physiological characteristics, such as photosynthetic rate (A), perspiration rate (E) and stomatal conductance (gs) in the Avaxi CL hybrid are negatively affected.

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