



## Article

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## DRIFT DISTANCE IN AIRCRAFT GLYPHOSATE APPLICATION USING RICE PLANTS AS INDICATORS

*Distância da Deriva em Aplicação Aérea de Glifosato Usando Plantas de Arroz como Indicadoras*

**ABSTRACT** - This study aimed to evaluate the potential for glyphosate drift during aerial application using rice plants as sentinels, aiming to determine the effect of drift on irrigated rice crops. For this purpose, a field experiment was performed using an entirely randomized design with four replicates, evaluating different distances from the site of application [control (no application), 0, 12.5, 25, 50, 75, 100, 150, 200, 300, and 400 m]. The experiment was carried out at the Granjas 4 Irmãos farm, located in the Rio Grande city, Rio Grande do Sul State, Brazil. The glyphosate dose application was 1,920 g e.a. ha<sup>-1</sup> (Roundup Transorb®, 480 g e.a. L<sup>-1</sup> glyphosate isopropylamine salt). A dose-response curve was developed to estimate the drift rate in sentinel plants, by applying increasing glyphosate doses in rice plants and assessing the injury level. The drift rates estimated by the injury level in sentinel plants were 14% (150 m), 13% (200 m), and 5% (400 m). Death of the experimental units was observed for distances between 0 and 50 m, while in distances between 75 and 150 m, 25 to 50% of the plants survived, reducing productivity. In the distances between 200 and 400 m, there was no reduction in productivity when compared to the control, even when the injury levels reached 52 to 82%. Thus, we concluded that a 5% glyphosate drift reached up to 400 m from the application range. Considering the recommendation of zero drift, distances greater than 400 m should be adopted to avoid symptoms in rice plants. We suggest using distances of more than 400 m in future studies.

**Keywords:** EPSPs injury, phytotoxicity, productivity, sub-doses.

**RESUMO** - O presente estudo teve como objetivo avaliar o potencial de deriva de glifosato durante aplicação aérea mediante o uso de plantas de arroz como sentinelas, a fim de determinar o efeito da deriva na cultura do arroz irrigado. Para isso, foi realizado um experimento em campo utilizando delineamento inteiramente casualizado com quatro repetições, avaliando diferentes distâncias [testemunha (sem aplicação), 0; 12,5; 25; 50; 75; 100; 150; 200; 300; e 400 m] a partir do local de aplicação. O experimento foi realizado nas Granjas 4 Irmãos, localizada no município de Rio Grande, RS. A dose de aplicação de glifosato foi de 1.920 g e.a. ha<sup>-1</sup> (Roundup Transorb®, sal de isopropilamina de glifosato 480 g e.a. L<sup>-1</sup>). Para estimação da taxa de deriva nas plantas sentinelas, foi elaborada uma curva de dose-resposta, aplicando doses crescentes de glifosato em plantas de arroz e avaliando o nível de injúria. As taxas de deriva estimadas mediante o nível de injúria em plantas sentinelas foram de 14% (150 m), 13% (200 m) e 5% (400 m). Foi observado morte de plantas nas distâncias entre 0 a 50 m, enquanto em distâncias entre 75 e 150 m a taxa de sobrevivência foi de 25 a 50%, causando redução na produtividade. Já nas distâncias entre 200 e 400 m

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*não houve redução na produtividade quando comparada com a testemunha, inclusive quando os níveis de injúria atingiram de 52 a 82%. Dessa forma, conclui-se que a deriva de glifosato de 5% atingiu até 400 m da faixa de aplicação. Considerando a recomendação de deriva zero, distâncias maiores que 400 m deverão ser adotadas para evitar sintomas em plantas de arroz. Sugere-se a utilização de distâncias maiores que 400 m em estudos futuros.*

**Palavras-chave:** EPSPs, fitotoxicidade, produtividade, subdoses.

## INTRODUCTION

Herbicide treatment in crops by aerial application is imperative in regions with frequent rainfall and wet soils (Reddy et al., 2010). During pre-sowing management of irrigated riceland, non-selective herbicides are necessary for the burndown of vegetation present in the areas. Among the options, glyphosate is preferably used. Similarly, in areas with soybean rotation, pre-sowing management begins with the total removal of the existing vegetation.

In the Rio Grande do Sul (RS) State, Brazil's leading rice producer, rice/soybean rotation areas have increased in the last decade. In 2009, the total cultivated area was 11,150 hectares, and during the 2016–2017 season, 297,453 hectares (IRGA, 2018). Almost all of these areas are seeded with glyphosate-resistant soybean cultivars due to the possibility of eliminating post-emergent weeds from the crops at a low cost (Duke and Powles, 2008).

As a result of pre-sowing management of rice and the pre- and post-emergence management of soybean, potential glyphosate drift events can threaten nearby areas with established rice. Environmental conditions during the application time are primarily responsible for the drift. The deposition of drops in the target area is affected by wind speed (Alves et al., 2017), air temperature (Arvidsson et al., 2011), flight height (Oliveira et al., 2013), and even fog (Crabbe et al., 1994). Regarding the technical factors during aerial applications, the high application speed (Van de Zande et al., 2005) and the flight height (Oliveira et al., 2014) are the most relevant factors that increase the drift rate.

Considering that glyphosate is a non-selective herbicide, its impact on the ecosystem results in the suppression of autotrophic organisms. Pesticide drift is a cause for concern due to public health impacts and contamination of adjacent crops and livestock by secondary compound residues (Benner et al., 2016; Borggaard and Gimsing, 2008). To overcome this problem, study using adjuvants aim to reduce the drift rate during applications, by increasing the diameter of the sprayed drop (Henry et al., 2015). Other study on wind tunnels estimated the potential for drift as a function of the type of spray tip and the drop size under ideal conditions and found that the drop size may vary as a function of the liquid to be sprayed (Ferguson et al., 2015).

Regarding aerial applications, experiment conducted with alpha-cellulose collectors found 5% drift rates at a distance of 30 m in the wind direction and around 0.5% of the dose at 150 m (Bird et al., 1996). Studies on the aerial drift with glyphosate and 93.5 L ha<sup>-1</sup> carrier volume estimated drift rates of 0.18 and 0.12 L of ha<sup>-1</sup> carrier volume from 160 and 320 m of the application area, respectively (Kirk, 2000). However, there are few works on the potential for glyphosate drift in the field, reporting that glyphosate drift may affect rice cultivation. Thus, the present study aimed to quantify the distance up to which glyphosate drift can occur during aerial applications, by determining the drift rate as a function of the range distance, and to evaluate the productivity of rice grains when submitted to aerial glyphosate drift.

## MATERIAL AND METHODS

The experiment was conducted in the field, at the Granjas 4 Irmãos farm, belonging to the group Joaquim Oliveira S/A, located in the municipality of Rio Grande, Rio Grande do Sul State, Brazil, during the 2017/18 harvest year. The experimental design was entirely randomized, with four replicates. The area for drift interception was delimited into lanes, where a 10 x 400 m sampling line was created, perpendicular to the flight, and aligned to the wind direction. The

aerial application was made on November 1, 2017, using an Ipanema aircraft with 10 Turboaero atomizers, equipped with a D10 flow restrictor, adjustment of the angle of the shovels in the position 3 and 205 kPa pressure, applying fine drops; this configuration was adopted to obtain excellent coverage during application. The aircraft flight was at 3-4 m height from the ground, with swath width 18 m. The aircraft applied eight strips while conducting the experiment. The application speed was 185 km<sup>-1</sup> (115 mph). The evaluated distances were as follows: control (no application), 0, 12.5, 25, 50, 75, 100, 150, 200, 300, and 400 m. The meteorological conditions during the application were 21.2 °C average temperature, 56% air humidity, and 8 km h<sup>-1</sup> average wind speed (with 2-15 km h<sup>-1</sup> gusts). The glyphosate dose applied corresponded to 1,920 g e.a. ha<sup>-1</sup> (Roundup Transorb®, 480 g e.a. L<sup>-1</sup> glyphosate isopropylamine salt).

The drift potential was determined in function of the resulting phytotoxicity level in sentinel rice plants. For this purpose, 4 L pots containing rice plants at the stage V3-4 from the IRGA 424 RI cultivar were used (Counce et al., 2000). The plants were collected at the 11<sup>th</sup> day before treatment from the rice production area belonging to the Granjas 4 Irmãos, removing them from the soil in blocks appropriate to the volume of the pots (4 kg) with cutting shovels. The quantity of seeds used in the farm during sowing was 90 kg ha<sup>-1</sup>. Fertilization was performed with 335 ha<sup>-1</sup> of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O fertilizer (05-20-20 formulation). The area where the plants were collected was previously treated with imazapyr + imazapic (63+21 g a.i. ha<sup>-1</sup>) in the pre-emergence of rice to keep it free from weeds.

The pots with sentinel rice plants were distributed in an area adjacent to the area destined for desiccation, according to the distances previously described, with four pots (replicates) per each distance evaluated. After aerial application, the pots were taken to a greenhouse. A severity scale was performed to determine the drift rate according to the phytotoxicity level, using rice plants and applying glyphosate doses of known concentration. The doses were applied on the same day of the aerial application; Subsequently, the plants were taken to the greenhouse along with the sentinel plants at the Universidade Federal de Pelotas (UFPEL), Capão do Leão, Rio Grande do Sul State, Brazil. The severity scale was determined using equation 1 below:

$$Y = Y_0 + \beta X_1 \quad (\text{eq. 1})$$

where Y corresponds to the injury level by visual analysis (0% = no injury, 100% = plant death); Y<sub>0</sub> is the initial value of phytotoxicity when X<sub>1</sub> = 0 (simulated drift glyphosate dose (%)), and β is the declining value corresponding to X<sub>1</sub>.

The concentrations of 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 3, 4, 5, 7.5, 10, 15, and 20% of the package insert dose applied in field (1,920 g e.a. ha<sup>-1</sup> Roundup Transorb®) were applied on plants in the pots. Injuries were visually assessed weekly. At the 22<sup>nd</sup> day after treatment (DAT), the plants submitted to aerial drift were compared with the plants submitted to simulated drift to relate the phytotoxicity levels and visually determine the glyphosate rate for each of the distances evaluated. Since the plants were collected at the V3-4 stage after the initial management of the farm, only the cover fertilizers and treatment of diseases and insects were required in the greenhouse. Plant management in the greenhouse followed the crop treatments of the Granjas 4 Irmãos farm, by applying 100 kg ha<sup>-1</sup> potassium chloride (0-0-60) on plants at the V4-5 stage, and 200 kg ha<sup>-1</sup> urea (46-0-0) equally distributed in two applications on plants at the V6 and R0 stages. A 10 cm water slide was kept for irrigation purposes. To prevent the appearance of diseases and insects in the crop, treatments were performed with 50 g a.i. ha<sup>-1</sup> azoxystrobin and 150 g a.i. ha<sup>-1</sup> tricyclazole fungicides, and 28 g a.i. ha<sup>-1</sup> thiamethoxam and 21 g a.i. ha<sup>-1</sup> lambda-cyhalothrin insecticides. Their application was made during the beginning of the flowering (R4 stage). The panicles were harvested by pot, and productivity was estimated (kg ha<sup>-1</sup>), relating the grain weight with the pot area.

An evaluation of the photosynthetic activity of plants was also carried out in the field at the 11<sup>th</sup> DAT, using the LI-6400XT infrared gas analyzer (IRGA), equipped with an artificial light source and CO<sub>2</sub> automatic injection. During the readings, the photon density was adjusted to 1,500 μmol mol<sup>-2</sup>s<sup>-1</sup>, with CO<sub>2</sub> injection in the 400 μmol mol<sup>-1</sup> chamber, 20 °C leaf temperature, and 500 μmol s<sup>-1</sup> airflow. Then, the photosynthetic rate (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and the carboxylation efficiency (CE) were determined. Plants less than 75 m away were disregarded from the IRGA analyses since the injury level did not allow the use of the equipment.

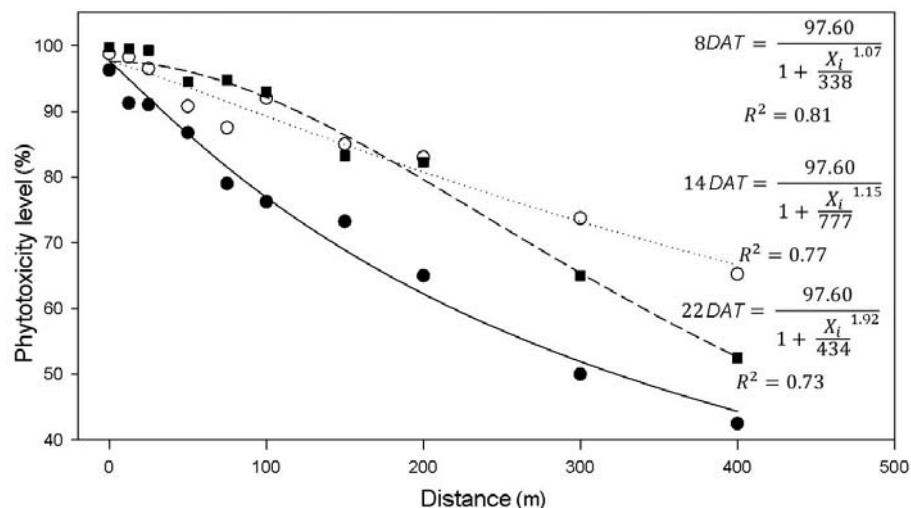
All data were submitted to analysis of variance (ANOVA) using the R statistical package (R Core Team, 2013). Data normality was verified using the Shapiro-Wilk test. The means for significant differences were compared by the Tukey test ( $P < 0.05$ ). A sigmoid logistic model of three parameters (equation 2) was adjusted to determine the phytotoxicity level in sentinel plants as a function of the distance from the application area of assessments made at the 8<sup>th</sup>, 14<sup>th</sup>, and 22<sup>nd</sup> DAT.

$$Y = \alpha / [1 + (X_i / X_{50})]^\beta \quad (\text{eq. 2})$$

where Y corresponds to the injury level by visual analysis (0% = no injury, 100% = plant death);  $\alpha$  is the highest value reported by the curve, representing the maximum value of phytotoxicity in sentinel plants;  $X_{50}$  is the distance from the application area where glyphosate drift would cause 50% phytotoxicity in sentinel plants;  $\beta$  corresponds to the declining value around  $X_{50}$ . The 95% confidence interval values were also calculated for the parameter  $X_{50}$  to compare them between the curves.

## RESULTS AND DISCUSSION

The ANOVA results showed a significant difference between the distances evaluated for the phytotoxicity level from the application area (Figure 1). According to the curves and equations of Figure 1, there was an increase in the phytotoxicity level when comparing the evaluations at the 8<sup>th</sup> and 14<sup>th</sup> DAT. The value of  $X_{50}$  (the distance required to cause 50% phytotoxicity) increased from 338 m (8<sup>th</sup> DAT) to 777 m (14<sup>th</sup> DAT). In this sense, on average, plants established at distances more than 338 m (95% CI, 286-390 m) showed a phytotoxicity level less than 50% at the 8<sup>th</sup> DAT after the drift occurrence, while plants established at a distance more than 777 m (95% CI, 473-1,081 m) had this same injury level in the second evaluation (14<sup>th</sup> DAT).

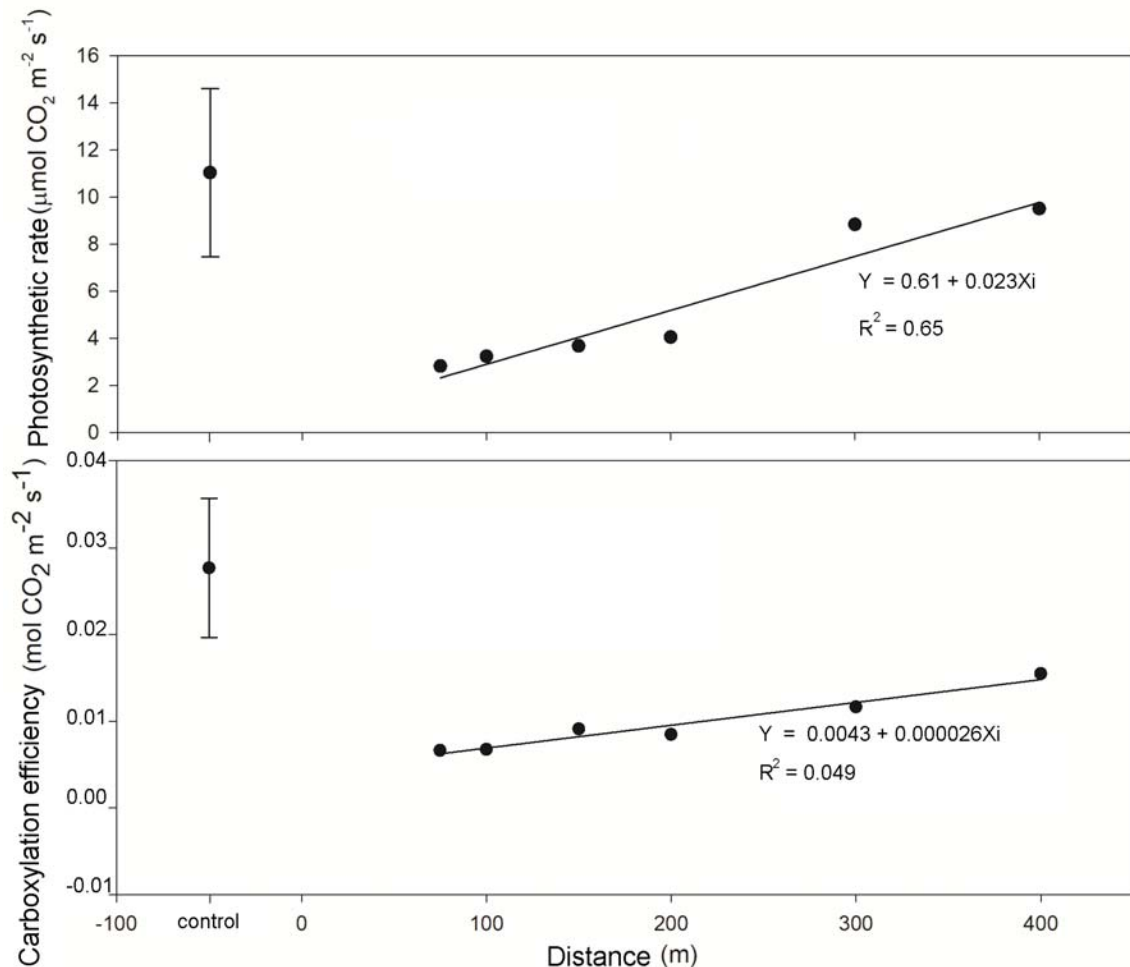


**Figure 1** - Visual evaluation of phytotoxicity levels at the 8<sup>th</sup> (●), 14<sup>th</sup> (○), 22<sup>nd</sup> (■) days after treatment (DAT), in response to aerial drift treatment with glyphosate. Capão do Leão, Rio Grande do Sul State, Brazil, 2017.

The phytotoxicity level at the 22<sup>nd</sup> DAT decreased compared to the 14<sup>th</sup> DAT with  $X_{50} = 434$  m (95% CI, 377-490 m), indicating that the plants started to recover after three weeks. This information is essential for evaluation of drifts in the field, where the data indicate that maximum phytotoxic effects will be observed approximately two weeks after the drift occurrence, thus helping in the decision-making regarding crop management (need for reseeded, extra fertilization or adjustment in irrigation, for example) and also in monitoring the evolution of the affected crop. The maximum phytotoxicity  $\alpha$  value (97.6%) in sentinel plants corresponds to plants at distances close to the application area (0-25 m). It should be noted that during the experiment, death was observed in all experimental units for distances less than 50 m, with 25, 25, and 50% survival rate at 75, 100, and 150 m distances, respectively. Thus, it is evident that aerial glyphosate

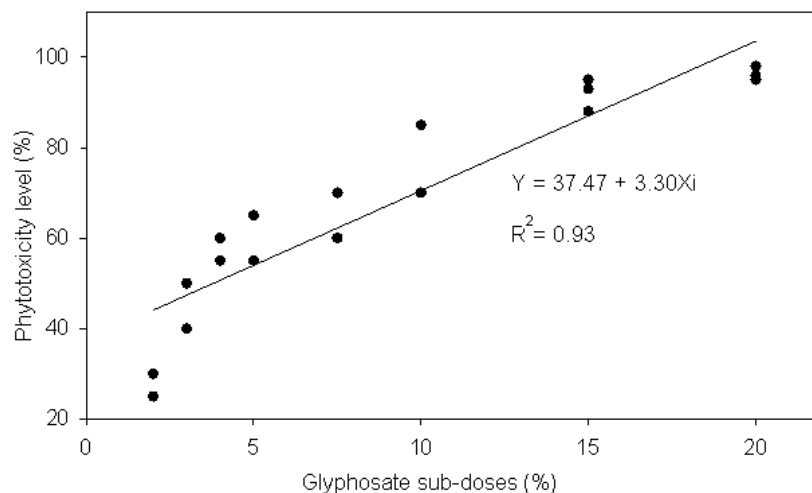
applications, even under favorable environmental conditions, irreversibly damage the rice crop located up to 50 m from the application range in the prevailing wind direction and result in a high mortality rate of plants up to a 150 m distance.

Differences in photosynthetic rate and CE were observed between the distances evaluated (Figure 2). Some studies have shown that the glyphosate affects stomatal conductance and reduces starch levels in the plant, suggesting that this herbicide affects carbon assimilation and photoassimilate translocation (Geiger et al., 1986; Geiger and Bestman, 1990). Stomatal closure (conductance) affects gas exchange and, consequently, CE. Similarly, the photosynthetic rate was reduced, presenting a significant difference for distances less than 200 m. The effect of glyphosate was more significant in distributed plants closer to the application range. Compared to the control, the CE in the plants was reduced by more than 50% for all distances evaluated. Studies with simulated glyphosate doses drift in sunflowers also report physiological changes in the photosynthetic rate (Vital et al., 2017).



**Figure 2** - Photosynthetic rate and carboxylation efficiency at the 11<sup>th</sup> DAT in rice plants submitted to aerial application of glyphosate. Error lines correspond to a 95% confidence interval. Capão do Leão, Rio Grande do Sul State, Brazil, 2017.

The phytotoxicity scale of Figure 3 was used to determine the drift rate, which reports the phytotoxicity level as a function of the glyphosate dose applied (simulated drift) to rice plants grown in a greenhouse. Using the phytotoxicity scale obtained from rice plants, the resulting drift rate in the field after aerial application of glyphosate can be estimated. For distances of 150, 200, and 400 m, the estimated drift rates were 14, 13, and 5% of the dose (1,920 g e.a. ha<sup>-1</sup> Roundup Transorb®), respectively. Figure 4 shows the comparison of plants from the aerial application in the field (sentinel rice plants) with those submitted to the application of simulated drift doses in the greenhouse.



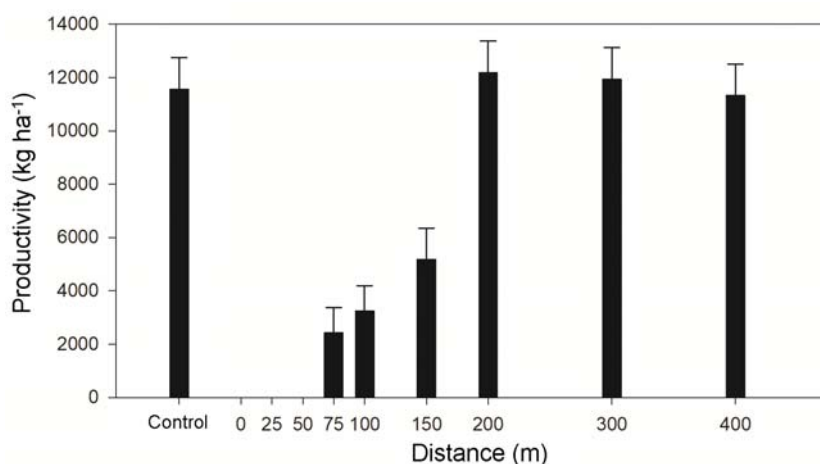
**Figure 3** - Phytotoxicity levels in rice plants submitted to glyphosate sub-doses at the 22<sup>nd</sup> DAT in a greenhouse. Capão do Leão, Rio Grande do Sul State, Brazil, 2017-2018 harvest.



**Figure 4** - Photographs of the experimental units. From left to right: control without application, sentinel at 150 m distance and simulated dose of 20% (A); control without application, sentinel at 200 m distance and simulated dose of 15% (B); control without application, sentinel at 400 m distance and simulated dose of 10% (C). Capão do Leão, Rio Grande do Sul State, Brazil, 2017.

Although there are field studies with rice that aimed to determine the losses in productivity due to the application of glyphosate sub-doses with drift simulation (Hensley et al., 2013; Koger et al., 2005), these studies had difficulties in establishing the dose to reach the plants in a real scenario since the drift doses vary from the point of application and, therefore, damage variability in a productive area is expected. On the other hand, the present study indicated the potential dose that sentinel plants received, as well as their production as a function of the distance from the application area, thus presenting unprecedented results.

Regarding productivity ( $\text{kg ha}^{-1}$ ), differences were observed between the evaluated distances. The means of the treatments are shown in Figure 5. For distances from 200 to 400 m, there was no difference in productivity in comparison with the control without the aerial application of glyphosate. The rice plants showed high recovery capacity because, even with observation of injury levels between 70 and 80% in the evaluation performed at the 14<sup>th</sup> DAT (Figure 1) and herbicide dose between 5 and 15% (Figure 3), there was no reduction in the plant production with distances more than or equal to 200 m from the application. In distances less than 150 m, there was the death of all plants (0-50 m) or a specific survival rate (75-150 m), causing a reduction in productivity. We suggest that continued management with cover fertilizers can help the plants to recover. Camargo et al. (2011) observed recovery of rice plants with high levels of injury by the application of post-emergence herbicides, suggesting that the management of the crop and the addition of nutrients by nitrogenous fertilizers (Golden et al., 2017), as well as the entry of water (Avila et al., 2009), contribute to plant recovery.



Column height = average of treatments; error bars = significant minimum difference (SMD) according to the T-test ( $P < 0.05$ ). \* All the plants found at distances from 0 to 50 m died. In distances of 75, 100, and 150 m, there were 25, 25, and 50% survival rate, respectively. From 200 m, the survival rate was 100%. Capão do Leão, Rio Grande do Sul State, Brazil, 2018.

**Figure 5** - Estimated productivity ( $\text{kg ha}^{-1}$ ) for sentinel rice plants submitted to aerial application of glyphosate.

The estimated drift rates using the phytotoxicity level of the sentinel plants were 14, 13, and 5% of the package insert dose ( $1,920 \text{ g e.a. ha}^{-1}$ ), corresponding to distances of 150, 200 and 400 m, respectively. Death and reduced productivity were observed in plants between 0 and 150 m of distance. In the distances between 200 and 400 m, there was no reduction in productivity when compared to the control, even with the observation of phytotoxicity levels of 52 to 82% in the evaluations.

The present results allowed to conclude that in aerial application, glyphosate drift can reach distances close to 400 m from the application site since the phytotoxicity level for this distance was 66% (14<sup>th</sup> DAT), and the herbicide dose was 5%. Concerning the safe application distance, although phytotoxicity levels of 80% at 200 m have been reported, the damage did not impact on productivity; it is therefore recommended to establish minimum ranges of 200 m from the target area. Considering the recommendation of zero drift, the distances of more than 400 m should be adopted to avoid symptoms in rice plants. We suggested the use of distances of more than 400 m in future studies.

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