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RESEARCH ARTICLE

Simulated drift of herbicides applied alone and in tank mix in grapevine

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Our goal was to evaluate the effects of simulated drift of clomazone e glyphosate applied alone or in a tank mixture over grapevines of Merlot variety. The experiment was set in a randomized blocks design, arranged in a 3x4+1 factorial scheme, with four replications. In factor A, the herbicides clomazone, glyphosate and the clomazone + glyphosate tank mixture was allocated and in B, doses of the same herbicides, representing drift simulations, of: 0, 25, 50, 75 and 100% of recommended doses. In 14, 28, 46 and 100 days after treatment, we evaluated the phytotoxicity of herbicides over grapevines. We determined the characteristics related to the increase of vegetative growth at the time of the experiment set-up and at the end of the experiment (100 DAT). At 10 and 20 DAT we also determined the net assimilation of CO_2 on grapevines that were treated with herbicides. The clomazone and the glyphosate applied either alone or in a tank mixture caused phytotoxicity in grapevine crops. The grapevine demonstrated to be sensitive, even to low herbicide doses, thus requiring caution when using pulverization of glyphosate, clomazone or the mixture of clomazone + glyphosate, in areas close to crops.

Highlighted Conclusion

1. Clomazone caused the least phytotoxicity in grapevines.
2. The highest phytotoxicity was observed in the application of glyphosate and the mixture of clomazone + glyphosate.
3. The grapevines demonstrated to be sensitive to sub doses of herbicides.

The cultivation of grapevine in the Southwest (SW) region of Rio Grande do Sul State (RS) has been rising considerably in the last years, mainly due to the edafoclimatic characteristics and the adaptability of cultivars in this region. However, this region is typically known for its irrigated rice cultivation and beef cattle, being the grapevine a marginal crop. In RS state, grapevine crops have a cultivation area of approximately 48,830 ha, in 2017 (IBGE 2018).

The RS state is the main rice producer in Brazil, with more than one million sowed hectares (CONAB 2017). The weed control in irrigated rice cultivations is done through the application of herbicides with different mechanisms of action, applied mainly with agricultural airplanes, with at least three applications during the cycle (SOSBAI 2016).

Aerial pulverizations have gained ground, especially in large cultivation areas (Lan et al. 2010), as it is the case of irrigated rice crops. This aspect is due to aerial applications does not lead to crop kneading and allow for the optimization of time and workmanship. However, when the technical recommendations for aerial pulverizations are not adopted, it may lead to problems in species not targeted for pulverization, that is, drift occurs in sensitive crops that are cultivated near the target crop (Gandolfo et al. 2013). The deposition of herbicides in non-targeted areas and the imminent possibility of damage has led to several researches involving the concept of simulated drift, mainly in cultures in which herbicide application is done with aerial equipment (Timossi and Alves 2001, Tuffi Santos et al. 2005, 2006, Wagner Jr. et al. 2008, Gandolfo et al. 2012, 2013, Foresti et al. 2015, Rodrigues et al. 2015).

The fruit producers from Southwest of RS state has been facing problems arising from drift of herbicides used in rice fields. The drift consists of wind dragging or even volatilization of small droplets of the pulverized herbicide syrup. Some factors must be considered during applications of pesticides to minimize drift effects, such as the products' physico-chemical characteristics, equipment type, calibration, pulverization tip type, applicator's expertise and meteorological conditions (Oliveira et al. 2013, Rodrigues et al. 2015).

The herbicides that are causing most problems of phytotoxicity in grapevines in the SW of RS state are clomazone and glyphosate. The clomazone belongs to the chemical group of isoxazolidinones, a group that inhibits the biosynthesis of carotenoids. It is absorbed by the plant's apical meristem and its translocation occurs through the xylem (phloem), following the transpirational flow. The injuries manifest in the youngest organs (Rodrigues and Almeida 2011). When absorbed by root and leaves, it blocks the synthesis of carotenoids, leading to photodegradation of chlorophyll and turning leaves white. It potentially causes drift when applied in pre-emergency, without soil incorporation (Oliveira Jr. 2011).

Regarding glyphosate, it belongs to the chemical group of substituted glycines, classified as non-selective, systemic and with a broad-spectrum action in weeds. It is predominantly absorbed by the chlorophyllated regions of plants (leaves and green tissues) and translocated mainly by the phloem to meristematic tissues (Rodrigues and Almeida 2011). Glyphosate is used for desiccation of vegetation, being a broad-spectrum agent (Bellé et al. 2014). However, low volumes of syrup have been used, which facilitates dragging of small droplets by the wind.

In addition to the isolated spraying of these herbicides causing damage to sensitive crops, rice producers in many situations make mixtures of clomazone + glyphosate in the pulverizer tank, which when applied improperly, can lead to even more losses to fruit producers. Gandolfo et al. (2012) relate that the tank mixture of herbicides, like glyphosate and the 2,4-D increase the possibility of drift in nearby fields.

Herbicide applications are done in October to November, a period in which grapevines are in reproductive stage; if drift occurs, it can cause chlorosis and leaf necrosis, foliar abscission, bunch abortion and/or lesions in branches (Oliveira Jr. et al. 2007), likely compromising the orchard and the fruit production in the coming years.

The damages caused by herbicide drift are recognized as a problem in orchards of different geographic regions (Wagner Jr. et al. 2008, Gravena et al. 2012). Even so, few works have evaluated herbicide drift effects in grapevines. Considering that the SW region of RS state is a prominent region for rice production and promising for an increasing grapevine cultivation, there is a need to evaluate the effects of drift of herbicides most commonly used by rice producers on the development and growth of grapevines.

Considering the information given above, we aimed with this work to evaluate the effects of simulated drift of clomazone and glyphosate applied alone or in a tank mixture over grapevines of Merlot variety.

MATERIAL AND METHODS

The experiment was conducted in an open environment in an experimental area in Itaqui, RS, Brazil (29°09'34.2"S, 56°33'15.9"W, altitude of 57 m) in the agricultural year 2011/12 e installed in plastic pots with capacity for 15 L of sieved, fertility-corrected soil, classified as Plinthudult.

Correction of soil fertility was performed following technical recommendations for grapevine cultivation (ROLAS 2004). The physico-chemical characteristics of the soil were: pH in water of 4.8; MO = 4.7 dag kg^{-1}; P= 6.8 mg dm^{-3}; K= 48 mg dm^{-3}; Al^{3+}= 0.5 cmolc dm^{-3}; Ca^{2+}= 4.76 cmolc dm^{-3}; Mg^{2+}= 1.03 cmolc dm^{-3}; CTC (t)= 6.4 cmolc dm^{-3}; CTC(T)= 15.6 cmolc dm^{-3}; H+Al= 9.7 cmolc dm^{-3}; SB= 60.59 cmolc dm^{-3}; V= 38% and Clay = 20%.

The experiment was set in an experimental block design with random treatments, arranged in a 3x4+1 factorial scheme, with four repetitions. In factor A were applied the herbicides clomazone, glyphosate and the tank mixture of clomazone + glyphosate and in B, doses of the same herbicides representing drift simulations of 0, 25, 50, 75 and 100% of recommended doses. The manufacturers' recommended doses for clomazone and glyphosate to control weeds in irrigated rice fields is 0.8 e 3.0 L ha^{-1} of commercial product or 500 and 1,440 g ha^{-1} of active ingredient, respectively. That way, the doses of clomazone, glyphosate and the mixture of both were 100% (0.8 and 3.0 L ha^{-1}), 75% (0.6 e 2.25 L ha^{-1}), 50% (0.4 e 1.5 L ha^{-1}), 25% (0.2 e 0.75 L ha^{-1}) e 0% (control without herbicide).

The herbicides were applied with a costal CO_2-pressurized pulverizer, with two jet pulverization tips, model DG 110,02, spaced 0.5 m into where it was pulverized syrup volumes of 150 L ha^{-1}. To avoid drift in adjacent plots, plastic curtains were used during application. We used the Merlot variety (*Vitis vinifera*), which is one of the most cultivated grapevines in the regions of Serra do Sudeste, Campanha and Fronteira Oeste of Rio Grande do Sul state.

At 14, 28, 46 e 100 days after treatment application (DAT), we evaluated the phytotoxicity caused by simulated drift in the vines, using a visual method. For such, two evaluators attributed percentage grades ranging from 0 to 100%, where 0% corresponds to no phytotoxicity and 100%, complete death of the plant, according to SBCPD methodology (SBCPD 1995).

The characteristics of rootstock diameter increment (IDTPE) and graft trunk diameter increment (IDTE) were obtained using a digital pachymeter, measuring the diameter in 5 cm below and above the exact grafting point, respectively. Regarding the height increase of plants (IAP) and crown volume (IVC), the data were obtained with the aid of a millimeter-graduated ruler. To IAP it was measured from ground level to plant apex, whereas for IVC it was measured the width (W), thickness (T) and height (H) of crowns, and the volume was calculated, using the formula (W x T x H). To determine characteristics related to the increments, the measurements were done in the moment of the experiment set-up and in the end of the experiment. These data were transformed into percentage increase relative to initial measurements.

It was also evaluated the photosynthetic rate (A - µmol m^{-2} s^{-1}), at 10 and 60 DAT with an infrared gas analyzer (IRGA), from ADC, model LCA PRO (Analytical Development Co. Ltd, Hoddesdon, UK), where in each block was evaluated under natural lighting in a day between eight and ten A.M. in clear sky conditions, so that the environmental conditions would be maintained homogeneous during the analysis.

The data obtained were tested for normality and homogeneity of residual variances by Lilliefors and Bartlett tests, respectively and later submitted to individual and joint variance analysis using the F test. When significant, we proceeded to regression analysis to the quantitative factor and Tukey's test to qualitative factor. The data expressed as percentage were transformed in arc sine of $(x/100)^{1/2}$. All tests were conducted at $p<0.05$, and analyzed using the statistical software ASSISTAT version 7.7 (Silva and Azevedo 2016).

RESULTS AND DISCUSSION

There was an interaction between the applied doses of herbicides on grapevines regarding phytotoxicity (Figure 1 and Table 1). It was observed that the phytotoxic effects of herbicides to grapevines were less pronounced in the first evaluation, at 14 days after treatment (DAT) and more pronounced at 28, 46 and 100 DAT (Figure 1). The increase in herbicide doses caused an overall rise in phytotoxicity in all evaluations. The glyphosate occasioned the biggest injuries, except at 14 DAT, when the mixture of clomazone + glyphosate showed a higher phytotoxicity, especially when 100% of the recommended dose was applied. The glyphosate is an herbicide which, depending on the species, can take from two to four weeks to cause phytotoxic effects on plants (Rodrigues and Almeida 2011), aspect also found in the present study.

In the highest doses applied, we observed that the new leaves of the grapevines sprouted withered, wrinkled and with visible symptoms of phytotoxicity caused by the herbicides. Leaf depigmentation (albinism) occurred in the application of clomazone, chlorosis or necrosis attributed to glyphosate and the depigmentation, chlorosis and necrosis for the tank mixture of both herbicides. In addition, it was observed fall of basal leaves for all treatments. According to Oliveira Jr. (2011), these symptoms are commonly observed when these herbicides are sprayed on plants.

When analyzing the glyphosate drift effects in yellow passion fruit trees, Wagner Jr. et al. (2008) observed from seven days after pulverization, symptoms similar to what was observed in the present work, with an increase of phytotoxicity as the applied dose increased. The change in the leaf coloring pulverized with glyphosate is related to chloroplast degeneration, decrease of the photosynthetic rate, nutrients concentration and dry biomass of shoot and root (Zobiole et al. 2010, Krenchinski et al. 2017). This aspect was also observed in other crops subjected to simulated drift of glyphosate, such as eucalyptus (Tuffi Santos et al. 2005), peach (Tuffi Santos et al. 2006) and cotton (Miller et al. 2004, Yamashita and Guimarães 2005). Timossi and Alves (2001) observed in orange trees, symptoms of phytotoxicity occasioned by clomazone in agreement with its expected mechanism of action, with formation of chlorotic spots in the younger leaves, that during development were coalescing until totally depigmented and leading to foliar abscission.

According to Rodrigues and Almeida (2011), the clomazone acts in the blocking of the synthesis of isoprenoid compounds, which are the precursors of photosynthetic pigments, leading to a decrease on carotene and phytol levels and, consequently, chlorophyll. Since carotenoids protect chlorophyll molecules from solar degradation, the mechanism of action of this product becomes bidirectional, inhibiting chlorophyll production and its protective pigments. Then, the leaves become discolored by lack of chlorophyll, dying in a short time period (Timossi and Alves 2001).

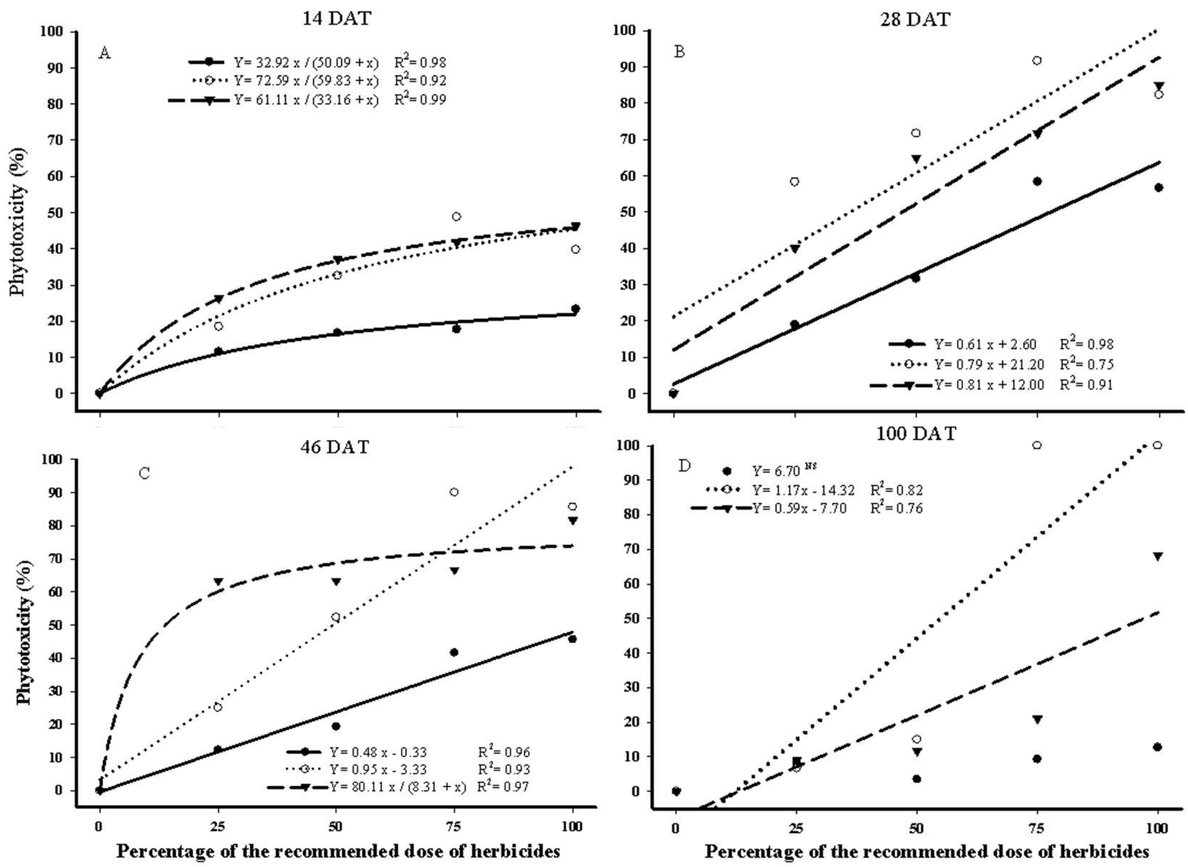

Figure 1. Phytotoxicity (%) in grapevines of Merlot variety in 14 (A), 28 (B), 46 (C) and 100 (D) days after treatment application (DAT), using percentages of 0, 25, 50, 70 e 100% the recommended dose of clomazone (●), glyphosate (○) and the tank mixture of clomazone + glyphosate (▼), simulating drift.

Table 1. Phytotoxicity (%) in Merlot grapevines at 14, 28, 46 e 100 days after treatment application (DAT) in function of administration of percentages of the recommended dose of herbicides employed in weed control in irrigated rice, simulating drift.

Herbicides	Percentages of the recommended dose of herbicides (%)				
	0	25	50	75	100
	14 DAT				
Clomazone	0.0 a[1]	9.8 c	12.3 c	17.7 b	23.3 c
Glyphosate	0.0 a	20.2 b	32.5 b	41.7 a	39.7 b
Clomazone + glyphosate	0.0 a	26.3 a	37.0 a	43.5 a	50.0 a
C.V. (%)	8.6				
	28 DAT				
Clomazone	0.0 a	21.0 c	31.7 b	58.3 c	56.7 b
Glyphosate	0.0 a	58.3 a	71.7 a	71.7 b	82.3 a
Clomazone + glyphosate	0.0 a	40.0 b	65.0 a	91.7 a	85.0 a
C.V. (%)	11.9				
	46 DAT				
Clomazone	0.0 a	12.3 b	19.3 b	41.7 c	45.7 b
Glyphosate	0.0 a	25.0 b	52.3 a	100.0 a	81.7 a
Clomazone + glyphosate	0.0 a	71.7 a	63.3 a	56.7 b	85.7 a
C.V. (%)	15.6				
	100 DAT				
Clomazone	0.0 a	8.0 a	5.0 b	9.3 c	12.7 c
Glyphosate	0.0 a	6.7 a	15.0 a	100.0 a	100.0 a
Clomazone + glyphosate	0.0 a	9.0 a	11.7 a	21.0 b	68.3 b
C.V. (%)	12.1				

[1] Means followed by different lowercase letters in the columns differ significantly from each other by Tukey test (p≤0.05).

At 14 DAT it was observed that the mixture of clomazone + glyphosate, compared to other herbicide administrations, caused, for all doses tested, the greatest phytotoxic effects, reaching roughly 42% of phytotoxicity when 100% of recommended dose was administered – 0.8 + 3 L ha^{-1} (Figure 1A). On the contrary, to the isolated application of clomazone it was observed a lesser phytotoxic effect, and regarding glyphosate alone, intermediate effects occurred (Table 1).

The simulated drift of clomazone, glyphosate e the mixture of both presented higher phytotoxicity up to 50% of recommended dose; in higher doses there were a stabilization of observed symptoms, even when 100% of the recommended dose was administered. We concluded that in vineyards affected by sub doses of these products will occur considerable injuries, since the mean phytotoxicity for the three herbicides in 50% of the recommended dose was 27.27% (Figure 1).

At 28, 46 e 100 DAT the highest phytotoxicities were caused by glyphosate at all doses, except at 46 DAT, in which the tank mixture of clomazone + glyphosate, with pulverization of up to 75% of recommended dose, caused the greatest injuries (Figures 1B to 1D). The glyphosate even when 75% of the recommended dose was administered, presented phytotoxicity rates of 71.7% at 28 DAT, and occasioned death at 46 and 100 DAT, these last in the highest doses (75 and 100%). As for clomazone alone, we observed minor symptoms in all doses and evaluations. At 100 DAT, to the highest dose administered (100% of recommended dose), there was a maximum phytotoxicity of only 12.7%.

The effects of injuries caused by clomazone were more intense in orange trees when sub doses of glyphosate were applied sequentially (Foresti et al. 2015), corroborating the present work, in which the herbicide glyphosate occasioned more pronounced effects than clomazone. Therefore, it is reinforced the importance to avoid aerial pulverizations, especially when using glyphosate for desiccation of vegetation before sowing rice or even the mixture of glyphosate + clomazone applied at the needle-point of rice or in any other culture to be sowed near vineyards because even in the lowest dose applied (25%), it was observed considerable injuries over grapevines (Figure 1 and Table 1). The drift of these herbicides to areas adjacent to the application site will lead to great injuries to grapevine crops, especially when the climatic conditions and application technology are not suitable to herbicide pulverization.

As mentioned, previous works related to glyphosate drift in fruit species, also observed that the herbicide caused reduction in plant growth or development (Wagner Jr. et al. 2008, Foresti et al. 2015). On the other hand, it is commonly described that the clomazone causes foliar abscission, chlorosis, chlorotic and necrotic stains in fruit skins (Timossi and Alves 2001), as well as decrease on leaf area, on chlorophyll content and in the dry mass of leaves and stem (Foresti et al. 2015).

There was no interaction between the tested treatments regarding the characteristics of rootstock diameter increment (IDTPE) and graft trunk diameter increment (IDTE), nor there were differences between the herbicides clomazone, glyphosate e clomazone + glyphosate to IDTE (Table 2).

Table 2. Graft trunk diameter increment (IDTE) in function of administration of percentages of the recommended dose of herbicides employed in weed control in irrigated rice, simulating drift.

Herbicides	Percentages of the recommended dose of herbicides (%)					
	0	25	50	75	100	Average
Clomazone	67.2 a[1]	63.2 a	56.8 a	59.9 b	41.7a	57.8 a
Glyphosate	67.2 a	29.8 b	67.4 a	60.3 ab	57.5 a	56.4 a
Clomazone + glyphosate	67.2 a	36.1 b	48.5 a	67.8 a	66.0 a	57.1 a
Average	67.2 A	43.0 B	57.6 AB	62.7 AB	55.1 AB	---
C.V. (%)	7.50					

[1] Means followed by different lowercase letters in the columns differ significantly from each other by Tukey test ($p \leq 0.05$).

Among the different doses applied, it occurred to IDTPE a linear descending behavior, that is, as the applied doses increased, there was a decrease in the trunk diameter increment (Figure 2A).

It was observed that the IDTPE in dose 0 (control), increased roughly 27%, whereas at the maximum dose of herbicides (100% of recommended dose) this increase was only of 19%, demonstrating that these herbicides are prejudicial to the growth and development of grapevines. For example, grapevines can present low sensitivity to 2.4-D, tolerating sub doses in the “half berry” stage, while in vegetative and flowering stages, can be very susceptible to these doses (Oliveira Jr. et al. 2007).

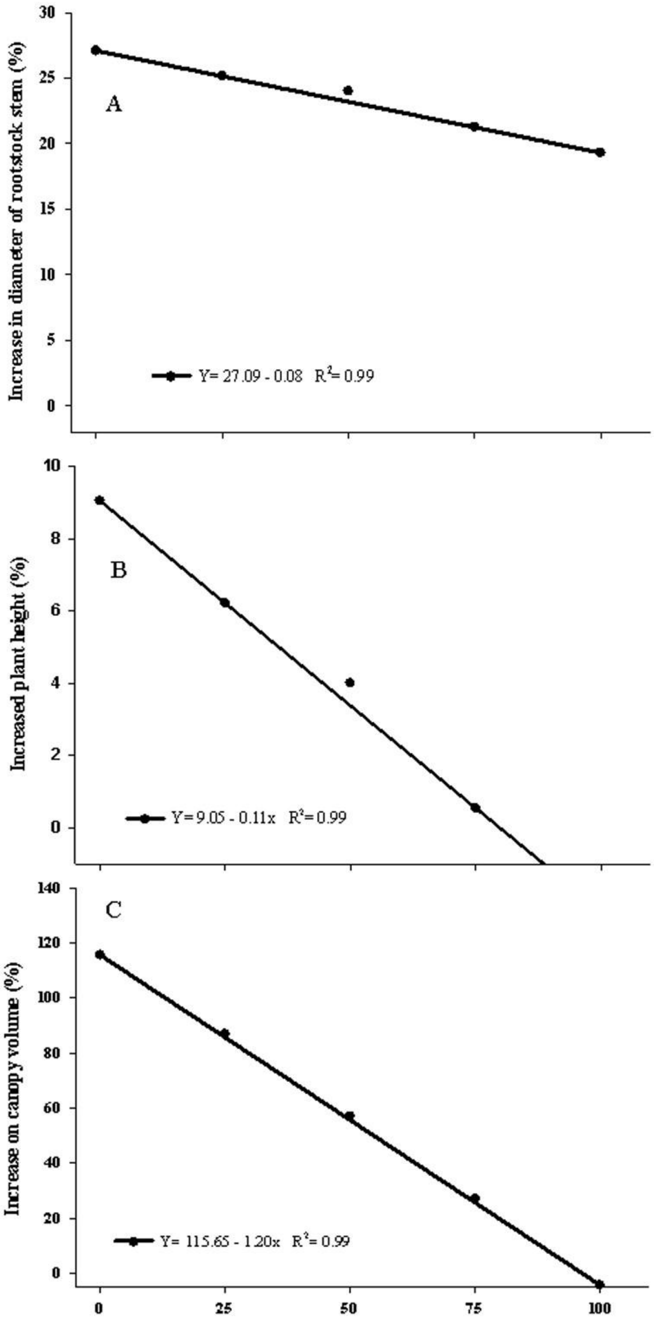

Figure 2. Percentage of increment of rootstock diameter - IDTPE (2A), of plant height – IAP (2B) and crown volume – IVC (2C) of grapevines from Merlot variety, in function of the mean of three herbicides (clomazone, glyphosate and the tank mixture of clomazone + glyphosate), simulating drift.

To the IDTE characteristic, the glyphosate and the mixture of clomazone + glyphosate, presented the smallest increments, of 29.8 and 36.1%, respectively. On the other hand, the same herbicides presented a higher IDTE than clomazone, at 75% of the recommended dose. In the mean of herbicides, it was possible to observe that the treatment applications at, 25, 50, 75 and 100% of recommended dose compared to the control, allowed for the decrease of IDTE (Table 2).

Generally, in studies of simulated drift, it's not always possible to observe the herbicide effect on the stem diameter. Costa et al. (2009) applied glyphosate in *Jatropha* plants and observed that the stem diameter presented a greater loss in doses of 180 and 360 g ha^{-1} (acid equivalent), with means of 10.8 e 31.5%, respectively, compared to the control without herbicide application. On the other hand, Wagner Jr. et al. (2008), applied glyphosate in passion fruit trees, and didn't observe the influence of simulated drift on stem diameter.

The increments of plant height (IAP) and crown volume (IVC) presented differences only for the herbicide doses with linear descending behavior, as the dose increased (Figures 2B and 2C). On the herbicide average, the maximum dose compared with the control provoked reduction of IAP in the order of 11.3%. As for IVC, when compared to the maximum dose relative to the control, the increment reduction was even higher, of 115%. (Figure 2C).

Results similar to the present work were verified to the culture of *Jatropha*, where Costa et al. (2009) observed an increase of growth paralysis of height and reduction of foliar area as doses increased, due to foliar abscission and interruption of apical growth. The crown volume is directly related to the foliar area; when this characteristic is affected, it can compromise the synthesis of assimilates, which are responsible to reserve accumulation in fruits (Oliveira Jr. et al. 2007).

At 60 DAT, the grapevines pulverized with 25, 50 and 75% and 25 and 50% of recommended doses of clomazone and glyphosate, respectively, presented a higher net assimilation of CO_2 than control grapevines (Figure 3). The plants that survived the application of clomazone and glyphosate with doses inferior than recommended, probably were in a repairing process from damage caused by the herbicides, because the glyphosate adversely affects photosynthetic parameters and chlorophyll fluorescence (Krenchinski et al. 2017). During the process of stress recovery, plants usually present a higher photosynthetic yield, to obtain the maximum amount of assimilates that will be synthesized in the repair of damaged tissues and growth of new vegetative structures.

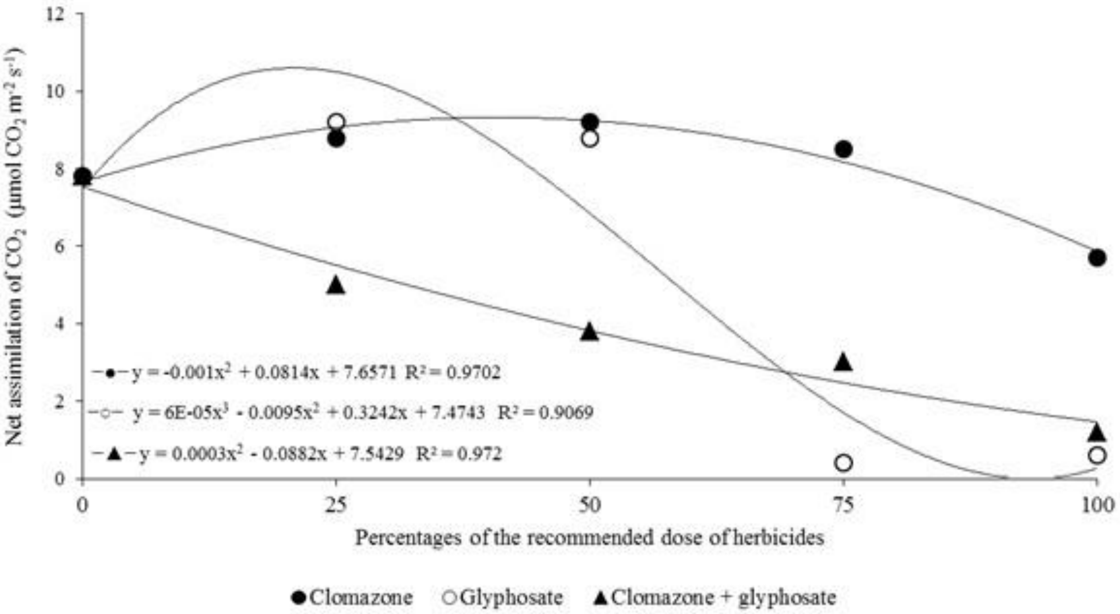

Figure 3. Net assimilation of CO_2 (μmol CO_2 m^{-2} s^{-1}) in grapevines from Merlot variety at 60 days after application of 0, 25, 50, 70 and 100% of the recommended dose of clomazone (●), glyphosate (○) and the tank mixture of clomazone + glyphosate (▲), simulating drift.

At 60 DAT with 75 e 100% of recommended dose of glyphosate and 100% of recommended dose of clomazone + glyphosate, there were the lowest net assimilations of CO_2, $\geq$ 1.2 μmol CO_2 m^{-2} s^{-1} (Figure 3). When herbicides were compared in simulations of 75 and 100%, the pulverization of clomazone presented a greater net assimilation of CO_2 than glyphosate alone and clomazone + glyphosate, so that glyphosate was more prejudicial to the grapevines (Table 3). These results corroborate the observations regarding phytotoxicity, in which the applications of glyphosate alone or in a mixture with clomazone, in the highest doses, led to more pronounced drift effects on plants.

Considering the effects of simulated drift of clomazone, glyphosate and the tank mixture of clomazone + glyphosate in grapevines from Merlot variety, it is possible to affirm that the herbicides led to a great phytotoxicity at the beginning of growth, which possibly affected the net assimilation of CO_2 and the distribution of assimilates for the increment of height, trunk diameter and crown volume. In this way, care must be taken regarding the technology of herbicide application to the control of weeds by rice producers, as well as the importance of following strictly the technical recommendations for aerial applications. We also recommend more studies which will provide technical information to avoid drift in vineyards.

The clomazone occasioned the lesser phytotoxicity in grapevines, but even so with considerable damage. The greater phytotoxicity were observed for the application of glyphosate and the mixture of clomazone + glyphosate. We conclude that the grapevine demonstrated to be sensitive to sub doses of herbicides, needing care when applying glyphosate, clomazone or the mixture of clomazone + glyphosate, in areas close to cultivation orchards.

Table 3. Net assimilation of CO_2 (µmol CO_2 m^{-2} s^{-1}) at 60 days after application of percentages of the recommended dose of herbicides employed in weed control in irrigated rice, simulating drift.

Herbicides	Percentages of the recommended dose of herbicides (%)					
	0	25	50	75	100	Average
Clomazone	7.8 a	8.8 a	9.2 a	8.5 a	5.7 a	8.0 a
Glyphosate	7.8 a	9.2 a	8.8 a	0.4 c	0.6 b	5.7 b
Clomazone + glyphosate	7.8 a	5.0 b	3.8 b	3.0 b	1.2 b	5.7 b
Average	8.0	7.6	7.3	4.0	2.6	---
C.V. (%)				15.6		

[1] Means followed by different lowercase letters in the columns differ significantly from each other by Tukey test ($p \leq 0.05$).

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