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Regression analysis to evaluate herbicide drift and injury in Roundup Ready cotton in wind tunnel[1]

Análise de regressão para avaliar deriva de herbicidas e injúria em algodoeiro Roundup Ready em túnel de vento

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ABSTRACT - The aim was: (i) to propose a regression model for the association between injury in Roundup Ready (RR) cotton plants and auxin herbicides drift, isolated or associated with glyphosate; (ii) to evaluate the effect of adding glyphosate to dicamba and 2,4-D auxinic herbicides on the physicochemical properties of the spray solutions; and (iii) to validate the wind tunnel as a tool to perform herbicide injury prediction. Three experiments were conducted using the following spray solutions: glyphosate (0.225 kg a.e. ha^{-1}); dicamba (0.120 kg a.e. ha^{-1}); 2,4-D (0.168 kg a.e. ha^{-1}); glyphosate + dicamba (0.225 + 0.120 kg a.e. ha^{-1}) and glyphosate + 2,4-D (0.225 + 0.168 kg a.e. ha^{-1}). Experiment I assessed drift in wind tunnel at distances of 5, 10 and 15 m from the nozzle spray drift. Experiment II assessed injury on cotton plants arranged at the same distances as in experiment I. Experiment III studied the physicochemical characteristics of the spray solutions used in experiment I, assessing surface tension, pH and density. The collected drift and injury are directly proportional, following a simple linear regression model, with increased drift and injury potential at 5 m from the sprayer. The addition of glyphosate to 2,4-D and dicamba herbicides reduced surface tension and pH of the spray solutions. The wind tunnel, besides evaluating drift potential, was found to be an alternative for the prediction of injury in RR cotton plants.

Key words: *Gossypium hirsutum* L.. Dicamba. EPSPS Inhibitors. Wind tunnel. 2,4-D.

RESUMO - Objetivou-se: (i) propor um modelo de regressão para a associação entre a injúria em plantas de algodoeiro Roundup Ready (RR) e a deriva dos herbicidas auxínicos, isolados ou associados ao glyphosate; (ii) avaliar o efeito da adição de glyphosate aos herbicidas auxínicos dicamba e 2,4-D sobre as propriedades físico-químicas das caldas de pulverização e (iii) validar o túnel de vento como ferramenta para realizar a predição de injúria de herbicidas. Foram conduzidos três experimentos, utilizando-se as caldas: glyphosate (0,225 kg e.a. ha^{-1}); dicamba (0,120 kg e.a. ha^{-1}); 2,4-D (0,168 kg e.a. ha^{-1}); glyphosate + dicamba (0,225 + 0,120 kg e.a. ha^{-1}) e glyphosate + 2,4-D (0,225 + 0,168 kg e.a. ha^{-1}). No experimento I foi avaliada a deriva, em túnel de vento às distâncias de 5, 10 e 15 m em relação à ponta de pulverização. No experimento II foi avaliada a injúria em plantas de algodoeiro dispostas nas mesmas distâncias do experimento I. No experimento III estudaram-se as características físico-químicas das caldas utilizadas no experimento I, com avaliações de tensão superficial, pH e densidade. A deriva coletada e a injúria são diretamente proporcionais, seguindo o modelo de regressão linear simples, com aumento do potencial de deriva e injúria aos 5 m de distância da barra de pulverização. A adição de glyphosate aos herbicidas 2,4-D e dicamba reduzem a tensão superficial e o pH das caldas. O túnel de vento além de avaliar o potencial de deriva, apresenta-se como uma alternativa na predição de injúria em plantas de algodoeiro RR.

Palavras-chave: *Gossypium hirsutum* L.. Dicamba. Inibidores da EPSPS. Túnel de vento. 2,4-D.

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INTRODUCTION

The evolution of weed biotypes resistant to glyphosate (5-enolpyruvylshikimate-3-phosphate synthase [EPSPS]) and other active ingredients have boosted the development of herbicide tolerant cultivars with other mechanisms of action, such as synthetic auxin (MONTGOMERY *et al*., 2017). Soybean genotypes *(Glycine max* L.) and cotton (*Gossypium hirsutum* L.) genetically modified for tolerance to 2,4-D or dicamba are commercially available (ALVES *et al*., 2017b). The modified cultivars perform detoxification via metabolization, determining the selectivity of these herbicides (RICHBURG *et al*., 2012).

The availability of cultivars tolerant to synthetic auxin expands the alternatives of resistant weed management and allows greater versatility in the use of these compounds (ALVES *et al*., 2017a), interfering in the productivity of certain crops (CULPEPPER *et al*., 2018). On the other hand, there may be an increase in particle dispersion to adjacent areas (SMITH *et al.,*2017) resulting from spray drift and compound volatility (CULPEPPER *et al*., 2018). Thus, plants that do not have resistance genes will be subject to injury (ROBINSON; SIMPSON; JOHNSON, 2013), mainly in the vegetative stage, when crops such as soybeans and cotton are extremely sensitive to these herbicides (EGAN; BARLOW; MORTENSEN, 2014). Plants of the Malvaceae family, such as cotton, are generally more sensitive to 2,4-D than to dicamba (EGAN; BARLOW; MORTENSEN, 2014; SMITH *et al*., 2017).

The use of glyphosate in mixture with auxinic herbicides contributes to increase the spectrum of application action, reduce the weeding community in more developed phenological stages (OSIPE *et al*., 2017), prevent and manage plant resistance to any of these herbicides (FLESSNER *et al*., 2015).

The occurrence of drift from auxinic herbicides can be influenced by several factors, including environmental factors (ALVES *et al*., 2017a; MUELLER; STECKEL, 2019a), and aspects related to the spray solution (CUNHA; ALVES, MARQUES, 2017; HAVENS *et al*., 2018; KALSING *et al*., 2018; OLIVEIRA *et al*., 2015; VIEIRA *et al*., 2018). In this context, the use of mixtures in herbicide tanks can affect the characteristics of the spray solutions and, consequently, the occurrence of drift (OLIVEIRA *et al*., 2019). The addition of glyphosate to the dicamba herbicide may increase the percentage of fine drops, depending on the physical-chemical properties of the spray solution and weather conditions, which favors the occurrence of drift (ALVES *et al*., 2017b).

Studies of drift potential are carried out in a wind tunnel, since it is a more accurate analysis due to the possibility of controlling meteorological elements such as temperature, relative humidity and wind speed (GANDOLFO *et al*., 2014). However, there is a need to evaluate the drift potential of auxinic herbicides, isolated or associated with glyphosate, under controlled conditions, and to verify the effect on crops, in order to validate models for predicting the potential risk of injury and provide information for evaluating strategies that minimize damage to susceptible crops.

The objective of this study was to: (i) propose a regression model for the association between the injury in Roundup Ready (RR) cotton plants, and auxin herbicides drift, isolated or associated with glyphosate; (ii) evaluate the effect of the addition of glyphosate to the auxinic herbicides dicamba and 2,4-D on the physical chemical properties of the spray syrup and (iii) validate the wind tunnel as a tool to perform the prediction of herbicide injury.

MATERIAL AND METHODS

Two wind tunnel experiments (I and II) and a laboratory experiment (III) between 2017 and 2018 were conducted.

Wind Tunnel Characterization

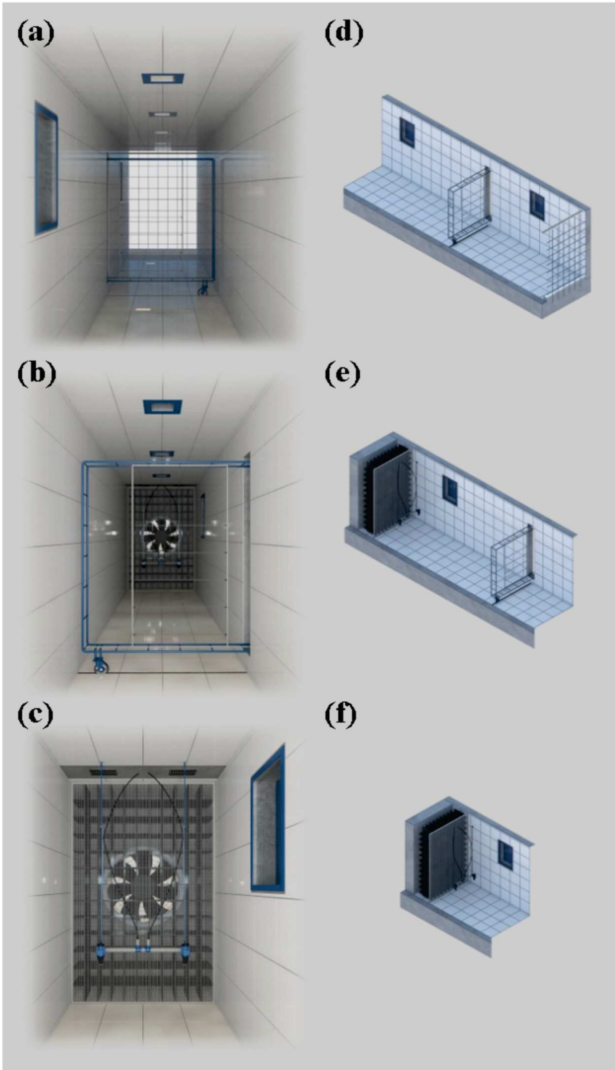
Experiments I and II were carried out in a wind tunnel with square cross-section 2 m of width and 20 m in length (Figure 1). The airflow was driven by an axial double-helix fan, with a diameter of 0,9 m, driven by the power take-off of a tractor (GANDOLFO *et al*., 2014).

The wind speed used in the tunnel was 2 m s^{-1}, being measured using a fan-type digital anemometer located inside the tunnel, 5 m from the spray bar, and at the vertical distance of 1 m from the tunnel floor. For the sprays, a system similar to an agricultural sprayer was used, composed of a reservoir with a capacity of 25 L, hydraulic circuit with pressure control and manometer.

On the inside of the tunnel was installed a spray bar (Figure 1c) composed of two nozzles spaced at 0.5 m, at the vertical distance of 0.5 m in relation to the floor and horizontal distance of 2 m from the deflector blades of the tunnel diffuser hive, which aims to standardize the direction of laminar air flow and, consequently, minimize the turbulence provided by the fan.

The spray tip used was flat jet with pre-orifice, model ADI 11002, with formation of medium class drops, working pressure of $4.14.10^5$ Pa and flow corresponding to 0.0152 L s^{-1}, equivalent to 100 L of spray solution ha^{-1}, using water as the application vehicle. In experiments I and II, the sprays were carried out for 120 seconds, under appropriate meteorological conditions for spraying, with air temperature below 30 ºC and relative humidity above 55%, recorded with a thermohygrometer. After the completion of each treatment, the spray system and the inside of the tunnel were washed with water before receiving the new spray solution.

Figure 1 - Wind tunnel representation: (a) Vertical drawers; (b) wires fixed in metal support positioned transversely to the direction of the tunnel airflow generated by the double-helix axial fan; (c) Spray bar at a horizontal distance of 2 m from the deflector blades of the tunnel diffuser hive. (d), (e) and (f) represent in 3D scheme the images (a), (b) and (c), respectively



Experiment I: Herbicide drift potential

The experiment was conducted in a completely randomized design, with four replications. The treatments were arranged in subdivided plots, and in the plots the spray solution and subplots were the collection distances of the drift. The spray solution were made up of glyphosate (0.225 kg a.e. ha^{-1}); dicamba (0.120 kg a.e. ha^{-1}); 2,4-D (0.168 kg a.e. ha^{-1}); glyphosate + dicamba (0.225 + 0.120 kg a.e. ha^{-1}); and glyphosate + 2,4-D (0.225 + 0.168 kg a.e. ha^{-1}). The products used were as follows: glyphosate isopropylamine salt (Roundup Original® SL, 0.360 kg a.e. L^{-1}), 2,4-D dimethylamine salt (DMA® 806 BR, 0.670 kg a.e. L^{-1}) and dicamba dyglycolamine salt (a suspension concentrate [SC] formulation, the basis of dyglycolamine salt, 0.480 kg e.a. L^{-1}). The drift collection distances were 5, 10 and 15 m in relation to the spray bar.

For the evaluation of the drift potential, the bright blue marker FCF 11.0% at the concentration of 0.006 kg L^{-1} was added to the spray solutions in order to measure the amount deposited. The determination of the drift was quantified by the marker, using collectors composed of five polyethylene wires of 0.002 m in diameter and 0.385 m in length, positioned at 0.30; 0.50; 0.70; 0.90 and 1.10 m in relation to the floor, fixed in metal supports, transversely to the direction of the tunnel airflow at each collection distance.

After spraying, the collector wires were stored individually in PVC tubes, 0.5 m long. Then, 0.025 L of distilled water was added to each tube and stirred manually, inversing them 180° for 15 times. The washing water of each tube was subjected to absorbance reading, using spectrophotometer (630 nm).

For the conversion of the absorbance values of each reading into marker concentration (mg L^{-1}), a standard curve was made with a sample of the spray solution of each treatment used and concentrations known by sequential dilution of the samples. With the absorbance values of each concentration and the standard curve of each treatment, the dye present in each sample from the collector wires was quantified. From the amount of marker and the volume of water used for extraction in the washing of the wires, the volume of spray solution (μL) collected in each wire was determined, being divided by the area of the wire (24.178 cm^2), to obtain the drift in μL cm^{-2}.

Experiment II: Herbicide injury in cotton

The experiment was conducted in a completely randomized design, with four replications, considering the same treatments used in experiment I. However, in the subplots, cotton plants were allocated for further evaluation of injury. For this, three seeds of the cotton crop FM 954 GLT were sown at a depth of 0.03 m in 1 L pots, filled with dystrophic Red Latosol. At seven days after emergence, thinning was carried out, maintaining one plant per pot.

In each repetition, two vases with cotton plants in V4 were positioned per tunnel drawer (Figure 1a), on the metallic supports of the collectors (Figure 1b), at a height of 0.30 m in relation to the tunnel floor, and at distances horizontal lengths of 5, 10 and 15 m in relation to the spray bar.

The evaluations of injuries were performed visually at 7 and 15 days after the application of treatments (DAT), on a percentage scale, in which zero and one hundred represent absence of injury and death of the plants, respectively. At 15 DAT, the plants were collected and dried in a greenhouse with forced air circulation at 60 ºC until they reached constant mass, in order to determine the shoot dry mass.

Experiment III: Physical and chemical properties of the spray solutions

For the evaluation of physical and chemical characteristics, the spray solutions used in previous experiments were again formulated in volumetric balloons of 1 L. Surface tension, density and hydrogenonic potential (pH) of the spray solutions were evaluated.

Surface tension (ST) was estimated by the gravimetric method by quantifying the non-cumulative mass of drops generated at the end of the volumetric burette of 0.05 L. In each repetition, 5 drops were evaluated, totaling 20 drops per treatment. The mass of the drops of the evaluated spray solutions was related to water droplets, considering the ST of $7.26.10^7$ N m^{-1}. The drops were deposited in a beaker on a precision scale. The ST of the spray solutions was calculated according to equation 1:

$$ST = \frac{MXST_{water}}{M_{water}} \tag{1}$$

Where: ST = surface tension (N m^{-1}) of the evaluated spray solutions; M = average mass of spray solutions drops; ST_{water} = surface tension of water ($7.26.10^7$ N m^{-1}) and M_{water} = average mass of water droplets. The density (kg m^{-3}) of each spray solutions was determined by weighing a 0.1 L solution on a precision scale, deposited in a volumetric balloon. The pH of each spray solutions was determined directly in the solutions through peagameter reading.

Statistical Analyses

To better understand the drift of the pulverized spray solutions in function to the displacement distance of drops of the spray tip used, the sum of the vertical wire drift was performed for each horizontal distance. Thus, the means of drift collected from the pulverized spray solutions, injuries, and shoot dry mass of the cotton plants were explained by regression models ($p<0.05$).

In experiment III, the assumptions of variance analysis were verified and then the analysis of variances was performed and compared with each other by the Tukey test ($p<0.05$). Pearson correlations were performed of the variables surface tension, pH, density, collected drift at 10 m, and injury at 7 and 15 DAT in the cotton plants due to dicamba spray solutions, 2,4-D, glyphosate + dicamba and glyphosate + 2,4-D sprayed in wind tunnel.

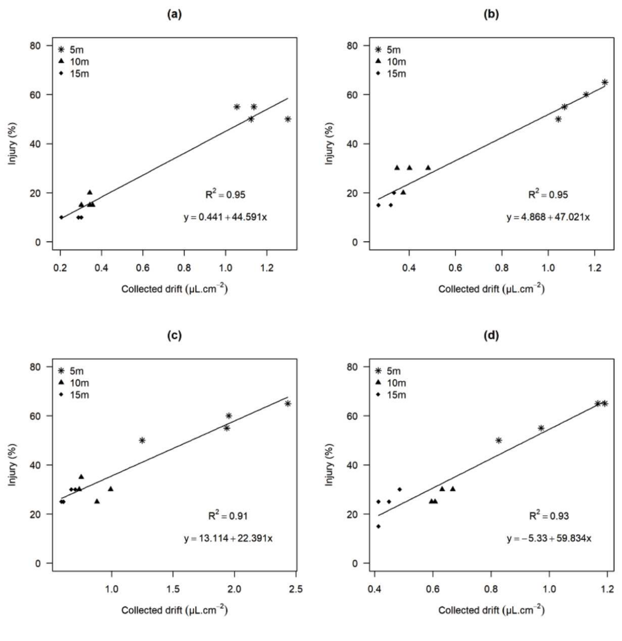
RESULT AND DISCUSSION

The analysis of simple linear regression of cotton plants injury in function to the drift of herbicide spray solutions and horizontal distances are presented in Figures 2 and 3, respectively. The linear regression model used shows a positive association. Thus, regardless of the pulverized spray solution, as the absolute values of collected drift increase, there is an increase in injury in plants. The wind tunnel was an effective alternative in predicting injury in RR cotton plants.

The isolated herbicide glyphosate did not cause injury in the crop since the cultivar used is tolerant to the herbicide. Thus, this treatment was disregarded from regression analyses. Other than the spray solution composed of glyphosate + dicamba (1.894 µL cm^{-2}), the means of the drift collected at 5 m away from the spray bar were less than 1.2 µL cm^{-2}, with a drift of 1.155; 1.130 and 1.039 µL cm^{-2} in spray solutions composed of dicamba, 2,4-D and glyphosate + 2,4-D, respectively (Figures 2 and 3).

At the distance of 15 m from the spray bar there was less drift collected. However, a significant percentage of injuries in leaf tissues was observed, regardless of the evaluation periods after the application of the treatments (Figures 2 and 3). From 7 DAT, regardless of the sprayed spray solution, only the drift collected at the horizontal distance of 5 m resulted in an average injury greater than 50% (Figures 3 and 4).

Figure 2 - Injury regression model (%) in cotton plants on 7 DAT in function of the collected drift (µL cm^{-2}) of herbicide spray solutions sprayed in a wind tunnel, at different collection distances in relation to the spray tip (m). (a) dicamba; (b) 2,4-D; (c) glyphosate + dicamba; and (d) glyphosate + 2,4-D



The cotton plants presented injury due to the drift of the sprayed spray solutions as early as the 7 DAT (Figure 2). The injury was characterized by purple spots on the leaf blade, accelerated growth of the adaxial part of the petiole compared to the abaxial, called hyponastic response, in addition to wilting, characteristic of synthetic auxin herbicides. These symptoms became even more evident at 15 DAT (Figure 3).

The averages of the dry mass of the shoot stems as a function of the injury from the drift collected at 5 m away from the spray bar were $1.55.10^{-3}$;$1.40\ .10^{-3}$;$1.46\ .10^{-3}$ and $8.8.10^{-4}$ kg plant^{-1} in spray solutions composed of dicamba, 2,4-D; glyphosate + dicamba and glyphosate + 2,4-D, respectively (Figure 4). The addition of glyphosate to 2,4-D resulted in a negative association with shoot dry mass, presenting the smallest magnitudes in the greatest distance in relation to the spray tip.

As for the physical and chemical properties of the spray solutions, there was a difference in the magnitude of the surface tension between the synthetic auxin and glyphosate herbicides, with the dicamba ($7.257.10^7$ N m^{-1}) and 2,4-D ($6.945.10^7$ N m^{-1}) showing greater surface tension than the isolated glyphosate ($4.477.10^7$ N m^{-1}). The addition of glyphosate to dicamba and 2,4-D reduced surface tension by 45.83 and 44.03%, respectively, when compared to spray solutions isolated from the same herbicides (Table 1).

Figure 3 - Injury regression model (%) in cotton plants at 15 DAT due to the collected drift (µL cm^{-2}) of herbicide spray solutions sprayed in wind tunnel, at different collection distances in relation to the spray tip (m), (a) dicamba; (b) 2,4-D; (c) glyphosate + dicamba and (d) glyphosate + 2,4-D

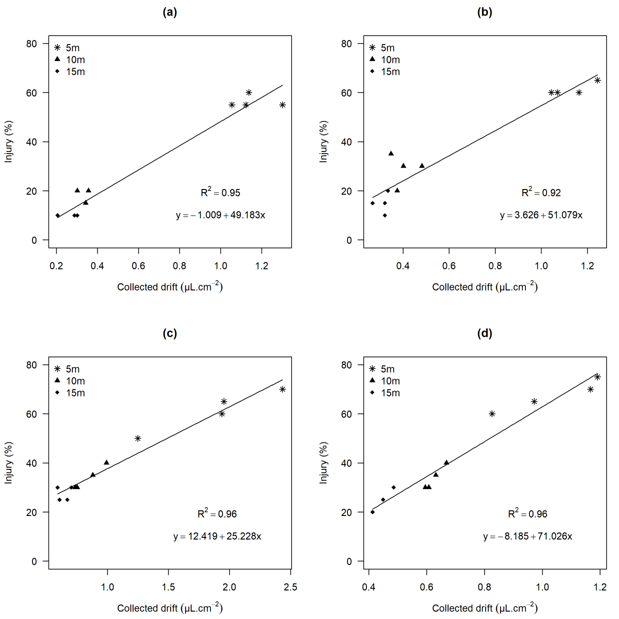


Figure 4 - Regression model of shoot dry mass (kg) in function of injury (%) in cotton plants at 15 DAT of herbicide spray solutions sprayed in wind tunnel, at different collection distances in relation to the spray tip (m). (a) dicamba; (b) 2,4-D; (c) glyphosate + dicamba and (d) glyphosate + 2,4-D

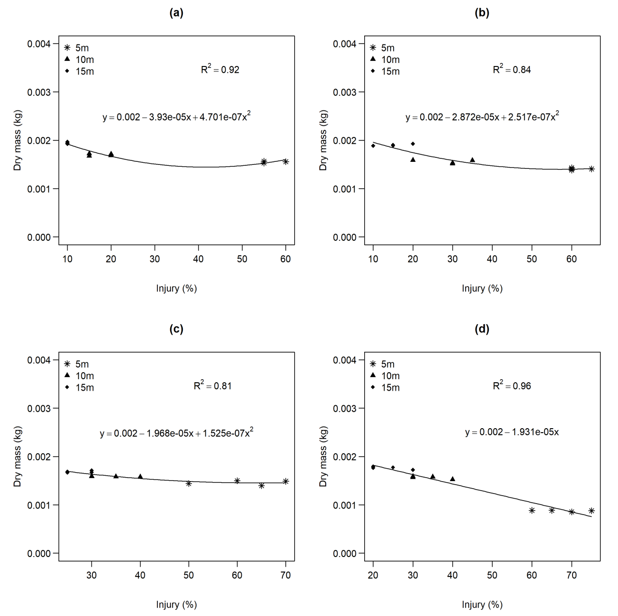


Table 1 - Physical and chemical properties of spray solutions sprayed in wind tunnel

Treatments	Surface tension (N m^{-1})	Density (kg m^{-3})	pH
Glyphosate	4.477.107 c*	99.88^{ns}	4.61 d
Dicamba	7.257.107 a	99.52^{ns}	6.02 a
2,4-D	6.945.107 b	99.55^{ns}	5.08 b
Glyphosate + Dicamba	3.931.107 d	99.88^{ns}	4.61 d
Glyphosate + 2,4-D	3.887.107 d	99.80^{ns}	4.76 c

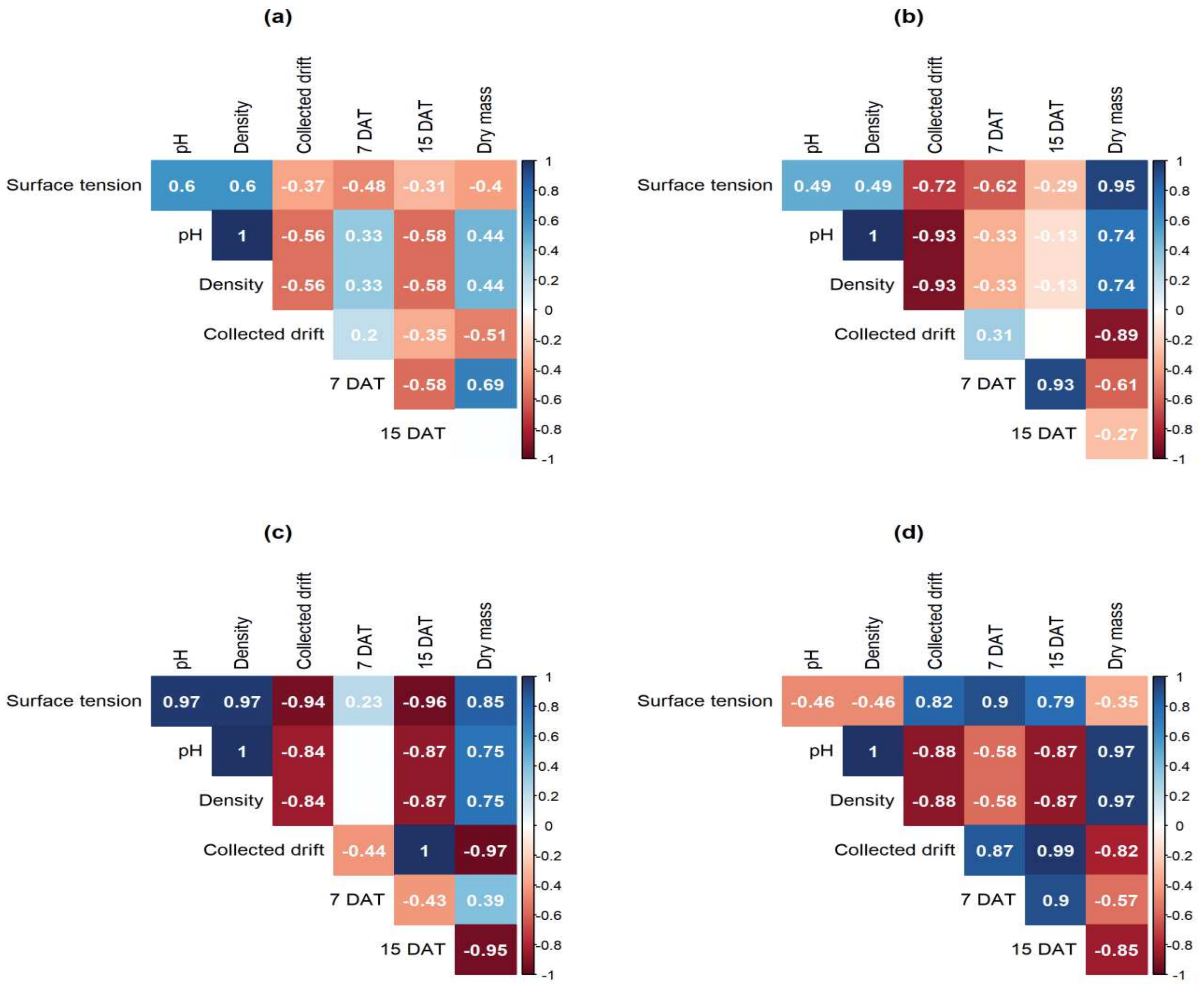
*The following averages of different letters differ by the Tukey test ($p<0.05$). ^{ns} not significant

The densities of the evaluated spray solutions did not differ from each other (Table 1). However, differences in pH values were observed. All spray solutions solutions evaluated presented pH below seven. The highest values were observed for dicamba (6.02) and 2,4-D (5.08) herbicides. However, the addition of glyphosate to these auxins resulted in a reduction in pH by 23.42 and 6.30%, respectively. The spray solutions formed only by glyphosate presented pH 4.61, not differing from the spray solutions containing the mixture of glyphosate + dicamba (Table 1).

The simple linear regression model is adequate to explain the injury as a function of the collected drift, which explains the positive correlation between the drift of the mixed spray solutions and the injury at 7 and 15 DAT, according to Pearson's correlation (Figure 5). At the greatest distances from the spray bar, there was less drift collected, however, the plants presented injury. Due to the herbicides used being systemic, the performance has less dependence on the distribution of drops on the leaf surface to cause injury, being possible to observe damage to leaf tissues even with a drift of less than 1.2 μL cm^{-2}.

The drift potential during the application of dicamba associated with glyphosate at 5 m of horizontal distance was the only treatment with drift above 1.2 μL cm^{-2}, which can be explained in function of the reduction of the surface tension and pH of the sprayed spray solutions (Table 1). The surface tension and pH of the spray solutions are determinant in the absorption of herbicides as a function of the electrolytic dissociation constant (pKa) (CUNHA; ALVES; MARQUES, 2017). These authors report that the surface tension of the synthetic auxin herbicides and ammonium salt glyphosate showed reduction in the

Figure 5 - Pearson correlation of the variables surface tension of the spray solutions (N m^{-1}), pH of the spray solutions, density of the spray solutions, drift collected at 10 m (μL cm^{-2}), injury (%) at 7 and 15 DAT and shoots dry mass (kg) of the cotton plant as a function of the herbicide spray solutions sprayed in a wind tunnel. (a) dicamba; (b) 2,4-D; (c) glyphosate + dicamba and (d) glyphosate + 2,4-D. *significant (p<0.05)



presence of surfactants, which influences the properties of the spray solutions, such as evaporation period, scattering and retention of drops on the surface of the biological target. Mueller and Steckel (2019b) observed that the addition of glyphosate reduced by 35.16% the pH of dicamba (ionizable organic acid), resulting in pH 5.09, which favored the occurrence of drift, possibly due to the volatility of dicamba after application, limiting the adoption of this mixture in tank.

In addition to the formulation, the combination of herbicides also provides different responses regarding the physical properties of the spray solution, such as the droplet spectrum (GRIESANG *et al*., 2017). This characteristic is directly related to the surface tension of the spray solution, which was reduced with the addition of glyphosate (Table 1). Alves *et al*. (2017b), reported that the addition of glyphosate to dicamba increases the percentage of drops below 100 μm, if compared to the isolated dicamba when sprayed with the standard XR flat jet tips and with AIXR air induction. These results demonstrate greater drift potential in the mixture of glyphosate + dicamba compared to their isolated applications. Dicamba dimethylamine salt provided a lower percentage of drops with a diameter of less than 141 μm than methylamine salt and dyglycolamine salt (VIEIRA *et al.,*2018).

In addition to the reduction of surface tension, the addition of glyphosate acidifies the spray solution containing 2,4-D and dicamba (Table 1). These synthetic auxins are herbicides with weak acid character, which have favored absorption in slightly acidic environments, due to the non-dissociation of molecules, which facilitates absorption by lipophilic cuticle (COBB; READE, 2010). Devkota and Johnson (2019) reported that the herbicide 2,4-D choline salt (0.456 kg a.e. L^{-1}) and the premixture 2,4-D choline salt + glyphosate dimethylammonium salt (0.195 + 0.205 kg a.e. L^{-1}) at pH 4.0 increased 10.5 and 9.6%, respectively, the control of *Conyza canadensis* compared to pH 6.5.

Another important aspect to be considered is the formulation of herbicides. The synthetic auxin herbicides and EPSPS inhibitors present differences in the physical and chemical properties between the salts present in the formulations. Kalsing *et al*. (2018) found that the formulation of 2,4-D dimethylamine salt showed higher drift potential than 2,4-D choline salt. The formulation of glyphosate isopropylamine salt used is of soluble concentrate type (SL) and thus contain surfactants in its composition which, although not compounding the active ingredient, can alter the spectrum of the spray solutions drops during spraying, making them thinner (HILZ; VERMEER, 2013) depending on the concentration used (OLIVEIRA; ANTUNIASSI; GANDOLFO, 2015). These surfactants present in glyphosate may have contributed to the injury rates caused by dicamba and 2,4-D due to the higher absorption of herbicides associated with adjuvants, providing greater penetration of the active ingredient sprayed in plants (Figures 2 and 3). Thus, the injury and shoot dry mass observed with the addition of glyphosate to 2,4-D and dicamba can be explained both by the occurrence of drift and by the greater absorption of auxins due to acidification of the spray solutions and/or presence of surfactants (Figures 2, 3 and 4).

Therefore, some care should be taken by producers in the use of synthetic auxins, with the aim of reducing drift and injury in adjacent areas, especially when in association with glyphosate. In these cases, it is recommended to use drops with higher volume median diameter (VMD), observe the direction and wind speed (0.83 to 3.33 m s^{-1}), adopt spray tips with air induction and add drift reducing adjuvants (ALVES *et al*., 2017a; BUTTS *et al*., 2019; CUNHA *et al*., 2016; VIEIRA *et al*., 2018).

CONCLUSION

The collected drift and the injury are directly proportional, following the simple linear regression model, with increased drift and injury potential at 5 m away from the spray bar. The addition of glyphosate to 2,4-D and dicamba herbicides reduces the surface tension and pH of the spray solutions. The wind tunnel, in addition to evaluating the drift potential, is an alternative in predicting injury in RR cotton plants.

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