Phytotoxicity and physiological changes in *Schinus terebinthifolius* Raddi under simulated 2,4-D drift and dicamba

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ABSTRACT

The use of auxin mimics herbicides in agriculture is widespread in a great diversity of crops. The indiscriminate use of herbicides can cause adverse effects in the plants. Among the plant species that stand out for being highly competitive and resistant to biotic and abiotic stresses, it is the brazilian peppertree (Schinus terebinthifolius Raddi.). The objective of this work was to evaluate the phytotoxic and photosynthetic alteration effects of different rates simulating the drift of the herbicides 2,4-D and Dicamba in brazilian peppertree seedlings. The experiment was carried out in a completely randomized design with four replications. The treatments were arranged in 2 x 8 factorial design (herbicide x doses). The factor herbicide consisted the herbicides 2,4-D and Dicamba, and, the factor doses eight percentages of the herbicide applied. Phytotoxicity and alteration of photosynthetic and fluorescence of chlorophyll a parameters were evaluated. Increased rate of the 2,4-D and Dicamba cause phytotoxicity to the plants, whose Dicamba promotes greater injuries. Dicamba was the herbicide that caused the greatest damage to the photosynthetic apparatus on brazilian peppertree plants, while for 2,4-D the plants showed higher recovery potential after herbicidal exposure.

Keywords: brazilian peppertree; auxin mimics; photosynthesis.

INTRODUCTION

The use of auxin mimics herbicides in agriculture is widespread, it is being used in pre-sowing, pre-emergence and post-emergence desiccation in tolerant crops. With the new traits incorporating resistance technologies to 2,4-D and Dicamba molecules, the use of these herbicides is expected to increase (Barret et al., 2016). The action of these herbicides results from the inability of the plant to metabolize them immediately, because they are able to acidify the cell wall of vegetables by the greater activity of the proton pump of ATPase, linked to the cell membrane (Senseman, 2007). Thus, the pH reduction in the apoplast region induced throughout the cell because of the greater activity of enzymes responsible for the loosening of cells (Usepa, 2013). The main effects are alteration of the cell wall plasticity, alteration of the protein content and increase in ethylene production (Grossmann, 2010). There is a decrease in auxin response genes, initiating a cascade of physiological responses within the plant, leading to plant death in sensitive dicots.

Even in less volatile formulations, the successive use of these herbicides may cause several effects. The main effect is drift after application, with phytotoxic effects on susceptible plants (Mortensen et al., 2012), such as tree species in native forest remnants and recovery plantations of degraded areas marginal to areas grown using agrochemical to control pests.

In Eucalyptus plants that receive drift of Dicamba suffered changes the physiological variables transpiration rate, internal and external CO2 concentration and stomatal conductance (Silva, 2020). As well drift of glyphosate also changed in photosynthetic rates, as observed in coffee

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(*Coffea arabica* L.) plants. The exposure to glyphosate underdoses caused different responses in the parameters of liquid photosynthesis, transpiration and stomatal conductance. These responses are more severe at higher doses and in newly transplanted plants (Carvalho *et al.*, 2012). The exposure to 2,4-D and Dicamba drift in cotton (Gossypium hirsutum L.), on the other hand, caused injuries to apples and seed-vessels, reducing yield (Smith et al., 2017).

The 2,4-D and Dicamba drift in communities of arthropods and forest vegetation caused a change in the population balance of several species (Egan *et al.*, 2014b). Considering the disturbance potential of herbicide application in both agricultural and forest areas, it is proposed to search for plant species tolerant to this type of stress. Among the plant species that stand out as highly competitive and resistant to biotic and abiotic stresses is, the Brazilian peppertree (Schinus terebinthifolius Raddi.). It is a species with potential for reforestation and recovery of degraded areas due to its wide geographical distribution in the tropics and competitive and growth capacity (Marcuzzo *et al*., 2015; Rorato *et al*., 2017). These peculiarities determine its presence in the composition of species in recovery projects in South America, as it produces a fast soil coverage.

The implantation of technologies of crop tolerant to the herbicides 2,4-D and Dicamba puts at risk the survival and the development of forest species located in areas close to crops due to the risk of drifts. Furthermore, it is likely that the application of these herbicides increases under these conditions. It can be carried out from October to February at the post-emergence period of tolerant crops. This period coincides with the establishment and initial growth of seedlings of tree species such as Brazilian peppertree cultivated in forest restoration plantations. In case of lack of care during the application of those herbicides, drifts may occur. In some studies, we found that the injury effect of Dicamba seen to be greater than 2,4-D in some crops (Egan et al., 2014a; Mohseni-Moghadam & Doohan, 2015). Therefore, the objective of this work is to evaluate the phytotoxicity and photosynthetic alterations in Brazilian peppertree seedlings by applying different doses of 2,4-D and Dicamba simulating drifts.

MATERIAL AND METHODS

Description and experimental design

The study was carried out from December 2018 to January 2019 at the Forest Nursery of the Federal University of Santa Maria (UFSM) in Santa Maria (RS State) (29º43'15'' S and 53º43'18'' W). The local climate is Cfa, humid subtropical, it characterized by well-distributed rainfalls throughout the year (Alvares *et al.*, 2013).

The experimental design was completely randomized with four replications. The treatments were arranged in a 2 x 8 factorial design (herbicides x doses). The factor herbicide consisted with use of 2,4-D and Dicamba, and the factor doses consisted of rates simulating drift, i.e., 0, 0.78, 1.58, 3.18, 6.25, 12.5, 25 and 50% of the label dose, considered as 835.7 g a.e. ha^{-1} and 600 g a.i. ha^{-1} for 2,4-D and Dicamba, respectively. These rates characterize the drift that may occur in plants. The application of doses was carried out using a backpack sprayer pressurized with CO_2, calibrated for an application volume of 150 L ha^{-1}, equipped with AIXR 110.015 nozzles and a working pressure of 35 lb in^2, with a pulverization height of 0.5m above the plant canopy. The temperature on the application day of herbices was 24 ºC, and the relative humidity (RU%) was around 83%, and wind with less 8 km h^{-1}.

The seedling of Brazilian peppertree was cultivated at 280-cm^3 plastic tubes filled with a peat-based substrate of *Sphagnum* and carbonized rice husk (3:1, v:v) fertilized with 6 g L^{-1} of controlled release fertilizer (15-09-12, NPK) (Osmocote, Brazil) which is well recommend to use in Brazilian peppertree (Cabreira *et al.*, 2017). The seedlings used had an average of 52.3 cm in height and 5.91 mm in stem diameter, considered of good quality and suitable for planting in the field (Araujo *et al.*, 2018). They were kept in full sun and irrigated at 9:30, 13:30 and 16:30 hours by micro-sprinkling (flow rate: 766 L h-1) during 15 minutes to avoid water stress.

2.2 Evaluation of phytotoxicity and physiological attributes

The phytotoxicity of plants of Brazilian peppertree was evaluated at 21 and 28 days after the application of treatments (DAT) at a percentage scale from zero to one hundred, where zero means the absence of injuries and one hundred means plant death (Frans, 1972). The physiological attributes were determined at 45 DAT between 08:00 and 11:00 hours (Souza et al., 2013). For the evaluations, completely expanded leaves from the upper third of the plants of four replications per treatment were used.

On that occasion, the following were determined net assimilation rate of CO_2 (A - µmol CO_2 m^{-2} s^{-1}), stomatal conductance of water vapors (Gs - mol H_2O $m^{-2}s^{-1}$), intercellular CO_2 concentration (ìmol CO_2 air mol^{-1}), transpiration rate (E - mol H_2O $m^{-2}s^{-1}$), water use efficiency (WUE - mol CO_2 mol H_2O^{-1}), obtained by the relation between the amount of CO_2 fixed by photosynthesis (A) and the amount of transpired water (E), and Rubisco carboxylation efficiency (A/Ci), obtained by the relation between the amount of CO_2 fixed by photosynthesis and the internal concentration of CO_2. These attributes were measured using a portable Infra-Red Gas Analyzer (IRGA), model LI-6400 XT (Li-Cor Bioscience, United States), using a photosynthetic radiation of 1,000 μmol m^{-2} s^{-1}, flow air volume of 400 μmols^{-1}, and CO_2 concentration of 400 μmol mol^{-1}.

The fluorescence emission of chlorophyll *a* was analyzed using a Junior-Pam portable modulated light fluorometer (Walz, Germany). Previously, the leaves were adapted to the dark for 30 minutes to measure the initial fluorescence (F_o) and, subsequently, subjected to a pulse of saturating light (10,000 μmol m^{-2} s^{-1}) for 0.6 s, thus determining the maximum quantum yield (F_v/F_m) of the photosystem II (PSII). The inhibition value due to the non-photochemical dissipation of the absorbed light energy (NPQ) was determined in each saturation pulse according to the equation NPQ = (F_m - F_m2)/F_m2 .

2.3 Statistical analysis

The data were analyzed using the software R (R development core team 2019) and Sigma Plot 12.3 (Systat software INC., United Kingdom), which it was also used to plot regression graphs. Previously, the data were subjected to normality test (Shapiro-Wilk) and homogeneity test (O'Neill-Matthews). After verifying the statistical significance, the data of the phytotoxicity variable were adjusted to the logistic type regression model according to the equation:

$$y = a/1 + (x/ED50)b \qquad \text{Eq. (1)}$$

where y = phytotoxicity (%); x = dose of herbicide (% of registration dose); *a*, x0 and *b* = parameters of the equation, where *a* is the difference between the maximum and minimum points of the curve, ED_{50} is the dose that provides a 50% response of the variable, and *b* is the slope of the curve.

Regarding the physiological attributes were adjusted to the cubic type regression model according to the equation:

$$y = y0 + ax + bx^2 + cx^3 \qquad \text{Eq. (2)}$$

Where y = physiological variable; y0 = intercept; x = dose of herbicide (% of registration dose); a, b and c the rate of change in variable.

RESULTS AND DISCUSSION

There was no need for data transformation due to lack of normality in any variable. The Brazilian peppertree showed phytotoxicity due to the use of the herbicides 2,4-D and Dicamba evaluated 21 and 28 DAT. The variables of phytotoxicity at 21 and 28 DAT interaction fitted the logistic regression model (Table 1, Figure 1). The injuries caused by Dicamba 21 DAT were greater than those caused by 2,4-D at the 1.56% dose of the experiment. The overlapping of confidence intervals of means and the significance of the mean test at the doses of 25 and 50% indicated that, according to the increase in the concentration of herbicide that reaches the plants, these differences tend to decrease. At the evaluation at 28 DAT, both herbicides showed a similar behavior and response, not overlapping from each other in all doses, except in 1.56% (Figure 1). In other hand, the estimate of the value of ED_{50} in the equations showed that the dose needed to promote 50% of the Dicamba response was lower than in 2,4-D for both periods of evaluation (Table 1), suggesting the greatest potential for damage of this herbicide in drift over Brazilian peppertree.

In an experiment evaluating the effects of Dicamba drift on soybeans, there was a linear behavior for herbicide phytotoxicity, however causing a phytotoxic effect similar to that found in this study at low doses (Egan *et al.*, 2014a). The simulation of 2,4-D and Dicamba drifts at different stages of white oak (*Quercus alba* L.) seedlings caused a greater phytotoxicity at the early stages of development, in addition to cause injuries to the growth points of the plants (Samtani *et al.*, 2008). It is noteworthy that this symptom was observed in Brazilian pepper tree plants for both herbicides already at the lowest doses tested in the present study, but mainly for the herbicide Dicamba. But in contrast, simulated drift of 2,4-D and Dicamba, has no negative effect on pecan [*Carya illinoinensis* (Wangenh.) K.Koch] yield in the nut-sizing stage (Wells *et al.*, 2019).

The results of the analysis of variance showed an interaction between the factors for the variables net assimilation rate of CO_2 (A), stomatal conductance of water vapors (Gs), intercellular concentration of CO_2 (Ci), transpiration rate (E) and water use efficiency (WUE) (Table 2, Figure 2). The assimilation rate of Brazilian peppertree plants subjected to the application of Dicamba showed a reduction due to the increase in herbicide doses in comparison to 2,4-D (Figure 2A). For this herbicide, there was an increase of A in the drift simulation at 25 e

Table 1: Logistic type regression parameters of phytotoxicity at 21 and 28 days after treatment (DAT), determination coefficient (R^2), values of the dose required to promote 50% phytotoxicity (ED50) of Brazilian peppertree in response to the application of different doses of 2,4-D herbicides and Dicamba

Herbicide	Equation	R^2
	Phytotoxicity at 21 DAT	
2,4-D	$y = 100.0/1 + (x/53.98)^{-1.66}$	0.98
Dicamba	$y = 80.42/1 + (x/21.96)^{-0.63}$	0.96
	Phytotoxicity at 28 DAT	
2,4-D	$y = 100.0/1 + (x/32.71)^{-0.45}$	0.86
Dicamba	$y = 100.0/1 + (x/14.37)^{-0.54}$	0.87

50% doses compared to the control without application, while Dicamba showed higher average only at control treatment. In a comparison among doses, we observed that 2,4-D presented a higher average at 0.78; 12.5 and 50% in the relation to Dicamba for A. The net assimilation rate of CO_2 is directly related to the CO_2 consumption of the environment and the production of biomass from the plants. The reduction of the photosynthetic rate has the consequence of lower carbon synthesis and, therefore, less biomass production will affect the growth and development of Brazilian peppertree plants.

The Gs, Ci and E parameters also showed reduced of averages when the plants were subjected to Dicamba compared to 2,4-D (Figure 2 B, C and D), the increase in the dose of Dicamba caused a reduction in these variables. The 2,4-D presented higher averages at 25 e 50% of the doses for Gs and E, while Ci presented at 50%. While Dicamba reduced, the Gs and E for all doses tested compared to control. In higher concentrations of these herbicides, cell division and the growth of meristematic tissues are inhibited, accumulating photoassimilates from the photosynthetic process (Hartzler & Anderson, 2018).

The stomatal conductance is the physiological mechanism that indicates the opening and closing of stomatal, adjusting this parameter is vital to avoid water loss and CO_2 capture, consequently affecting the intercellular CO_2 concentration as well as the transpiration of plants. The Ci in the leaf mesophile is reduced by stomatal closure, with a consequent decrease in the rate of carbon dioxide assimilation (Jadoski *et al.* 2005). As well the transpiration process is directly related to the regulation of stomatal opening and closing, so the smaller the stomatal opening process, the lower will be the transpiration of plants due to higher stomatal resistance. Therefore, the Gs regulates the input flow of CO_2 and water output by the stomata (Taiz *et al.*, 2017).

So the responses of net CO_2 assimilation rate, the stomatal closure, the intercellular CO_2 and transpiration rate were due the application of Dicamba and 2,4-D herbicides, but the mostly reduction of the variables it was due to Dicamba that presented be the herbicide with more negative effects to the plants. This may be occur due to the fact that they auxin-mimicking herbicides act in several physiological processes, such as stimulation of ethylene production, which also stimulates abscisic acid production, accumulating in the plants which in turn also stimulates the synthesis of abscisic acid (ABA), accumulating initially in the leaves and after will be translocated by the plant and acting negatively on the stomatal closure, limiting the net CO_2 assimilation rate and consequently reducing the biomass production of the plants (Mercier, 2004).

The WUE in plants submitted to the application of Dicamba did not show any differences among the doses of this herbicide, whereas for 2,4-D there was a reduction of WUE with in the higher herbicide dose 50% (Figure 2E). At comparison between doses, Dicamba presented higher WUE than 2,4-D at all doses. This may occur because the greater efficiency of water use is related to the shorter opening of the stomata, since this opening provides CO_2 uptake for photosynthesis and water loss through transpiration (Pereira-Netto, 2002), and we can observed in our results that Dicamba was the herbicide reduced the Gs and Ci, due this the plants presented higher WUE.

The increase of variable A/Ci was higher when increased of herbicides doses (Figure 3 A). Dicamba showed lower A/Ci compare to 2,4-D (Figure 3B). A/Ci is

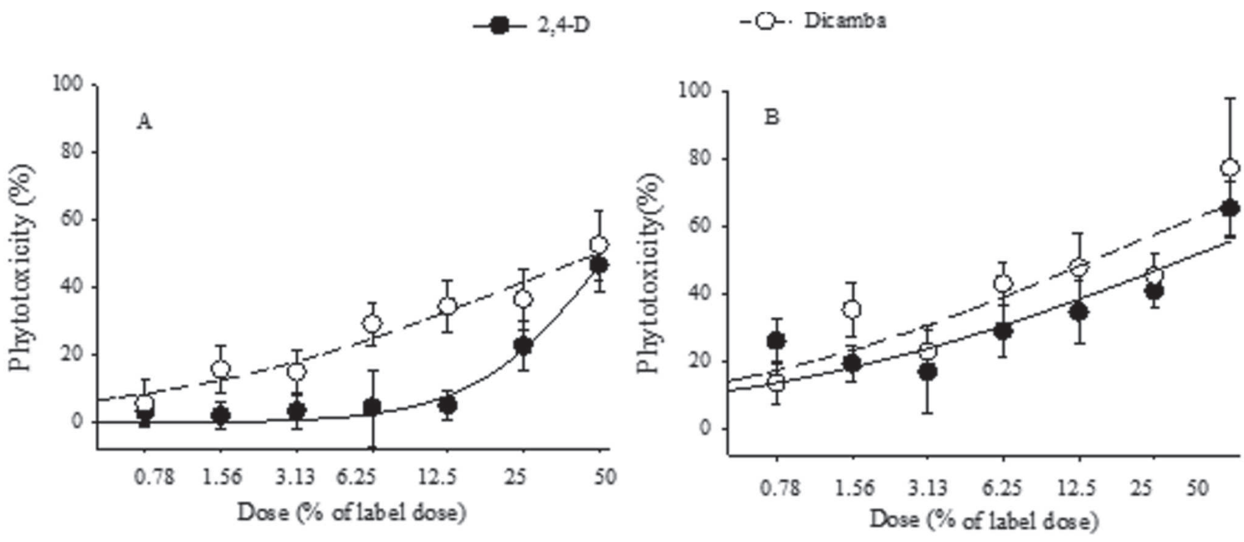

Figure 1: Phytotoxicity (%) in Brazilian peppertree plants submitted to doses of 2,4-D and Dicamba at 21 (A) and 28 (B) days after application of treatments.

closely related to Ci and A (Machado *et al.* 2005). In this sense, the increase observed in the Rubisco carboxylation efficiency, in the present work, is due to the increases registered in the internal concentration of carbon dioxide and to the gains in the CO_2 assimilation rate, observed mainly when the herbicide 2,4-D was used.

The risk of drifts resulting from the application of herbicides to other non-target crops depends on the carrier water rate, the dose, and the exposure time of plants (Egan & Mortensen, 2012; Hensley *et al.*, 2012). In general, Brazilian peppertree plants have a greater capacity than Dicamba for recovering physiological activity when there is a drift of 2,4-D. This probably occurred due to the ABA produced when these herbicides are applied. It acts by reducing the turgor pressure, closing the stomata to reduce stress and, consequently, reducing the photosynthetic activity of plants (Grossman, 2010).

The concern about the movement of these herbicides out of the application target, causing phytotoxicity in non-target crops, generates great discussions over the use of 2,4-D and Dicamba (Egan *et al.*, 2014a). Our results showed that both herbicides caused injuries Brazilian peppertree plants with a simulated drift, however, Dicamba showed greater phytotoxicity and reduced physiological parameters compared to 2,4-D. In a simulation of Dicamba drift in soybean culture, there was a greater negative effect on productivity compared to 2,4-D (Silva *et al.*, 2018). At low doses, these herbicides have hormonal properties similar to natural auxins, it promotes plant growth; at high doses, it promotes overgrowth of plants, including shrinkage and leaf fragility, stem curling, and general abnormal growth (Velini *et al.*, 2010; Grossman, 2010).

The results of the analysis of variance showed an interaction between the factors for the variables chlorophyll *a* fluorescence, initial fluorescence (F_o), maximum quantum yield (F_v/F_m) of the PSII, and non-photochemical quenching (NPQ). The attributes F_o, F_v/F_m), NPQ evidenced that the increase in doses of 2,4-D and Dicamba changed the photochemical efficiency of Brazilian peppertree plants (Figure 3).

The loss of energy by F_0 increased significantly at 3.18; 6.25; 12.5 and 25% doses of the Dicamba, whereas the 2,4-D had a higher F_0 at doses of 25 and 50% (Figure 3C). The doses only had differences at 1.58; 3.18; 6.25; 12.5 and 50%. In general, plants under the effect of Dicamba showed higher values of F_0 in relation to those with simulated 2,4-D drift at 0.78; 3.18; 6.25; 12.5 and 50% of the dose. For 2,4-D the higher F_0 was higher at 50% of de label dose.

Regarding this greater drift, the use of photochemical energy in plants with 2,4-D was lower than the treatment without application of herbicide. With the exception of the highest simulated drift dose, the negative effects observed for F_0 under Dicamba application were more pronounced in relation to 2,4-D, whose greatest photochemical energy losses were observed when 3.13% of the registered dose was applied (F_0 = 144.3).

The F_0 represents the initial fluorescence, corresponding to the fraction of the energy absorbed by the antenna complex and is not transmitted, and therefore is not absorbed by photosynthetic pigments (Rascher *et al.*, 2000).

The value of F_0 may increase when the centers of reaction of photosystem II are compromised or to transfer of the excitation energy from the antenna to the reaction centers is impaired (Cruz *et al.*, 2009), so the increase of this parameter means that both herbicides cause stress in Brazilian Peppertree plants.

The increase in herbicide doses caused a significant reduction in Fv/Fm in leaves of Brazilian peppertree. The lowest values of Fv/Fm were observed in seedlings grown at the 25 e 50% doses at 2,4-D herbicide. For Dicamba in

Table 2: Cubic type regression physiological attributes, of Brazilian peppertree in response to the application of different doses of 2,4-D herbicides and Dicamba

Herbicide	Equation	R^2
	A (mmol CO_2 m^{-2} s^{-1})	
2,4-D	$y = 9.82 - 2.86x + 0.47x^2 - 0.01x^3$	0.62
Dicamba	$y = 9.16 - 4.0x + 0.84x^2 - 0.05x^3$	0.72
	Gs (mol H_2O $m^{-2}s^{-1}$)	
2,4-D	$y = 0.10 - 0.01x + 0.01x^2 - 0.001x^3$	0.87
Dicamba	$y = 0.13 - 0.06x + 0.01x^2 - 0.001x^3$	0.79
	Ci (mmol CO_2 ar $^{mol-1}$)	
2,4-D	$y = 207.07 - 57.6x + 15.07x^2 - 1.18x^3$	0.86
Dicamba	$y = 267.72 - 13.37x + 1.33x^2 - 0.05x^3$	0.79
	E (mmol H_2O m^{-2} s^{-1})	
2,4-D	$y = 1.71 - 0.31x + 0.15x^2 - 0.01x^3$	0.83
Dicamba	$y = 2.66 - 1.09x + 0.22x^2 - 0.01x^3$	0.74
	EUA (mol CO_2 mol H_2O^{-1})	
2,4-D	$y = 5.27 - 1.17x + 0.382x^2 - 0.02x^3$	0.80
Dicamba	$y = 3.55 - 0.329x + 0.09x^2 - 0.01x^3$	0.21
	A/Ci	
Doses	$y = 0.04 - 0.01x + 0.003x^2 - 0.0002x^3$	0.89
	F_0	
2,4-D	$y = 83.15 - 0.49x + 0.08x^2 - 0.001x^3$	0.77
Dicamba	$y = 95.37 - 9.15x + 0.43x^2 - 0.005x^3$	0.52
	Fv/Fm	
2,4-D	$y = 0.64 - 0.01x + 0.0005x^2 - 0.000007x^3$	0.59
Dicamba	$y = 0.63 - 0.02x + 0.001x^2 - 0.00002x^3$	0.34
	NPQ	
2,4-D	$y = 0.16 - 0.009x + 0.0004x^2 - 0.000008x^3$	0.89
Dicamba	$y = 0.14 - 0.009x + 0.00006x^2 - 0.000002x^3$	0.79

general the increase of doses reduced Fv/Fm (Figure 3D). In comparing doses, it is not found differences between 2,4-D and Dicamba, except on 3.18% dose. On the other hand, plants that did not receive herbicide showed the highest quantum yield (0.74). For the quantum yield of FSII, plants that are not in a state of stress, the values should vary between 0.75 and 0.85 (Araus & Hogan, 1994). While all doses caused reductions of Fv/Fm with the use of 2,4-D and Dicamba in comparative relation to the zero dose (Figura 3D), so the use of herbicides caused a stress situation.

The NPQ increased considerably with the simulation of 2,4-D and Dicamba drifts. The highest values were obtained when plants were submitted to the two highest

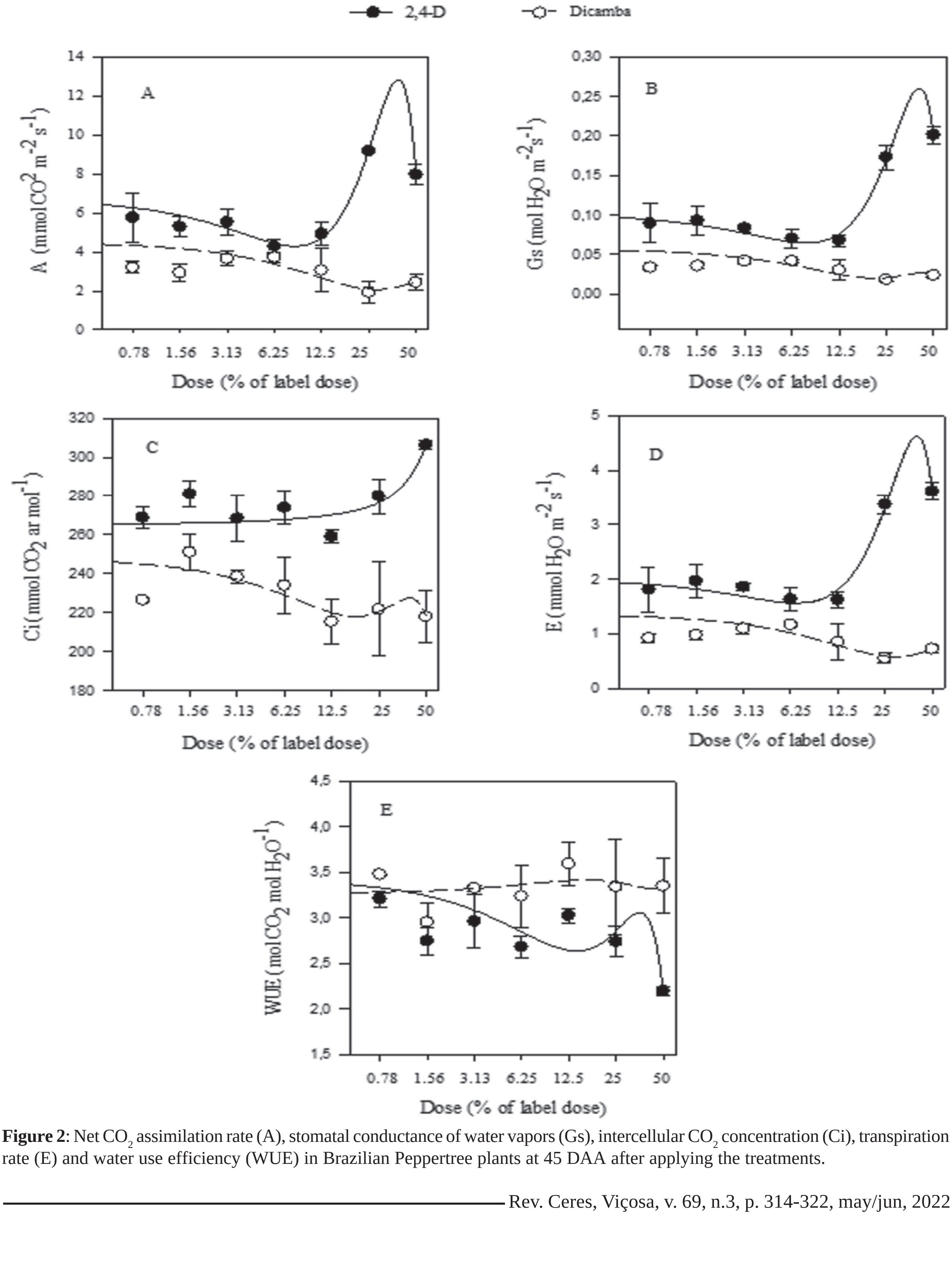

Figure 2: Net CO_2 assimilation rate (A), stomatal conductance of water vapors (Gs), intercellular CO_2 concentration (Ci), transpiration rate (E) and water use efficiency (WUE) in Brazilian Peppertree plants at 45 DAA after applying the treatments.

doses of herbicides, represented by 25 and 50% of the registration dose (Figure 3E). The use of 2,4-D was proportional to the highest NPQ in relation to Dicamba. The application of 25 and 50% of the dose 2,4-D presented higher NPQ than Dicamba.

The higher values of F_0 demonstrate that the seedlings present a greater damage in the reaction center of the PSII and they have a reduced transfer of excitation energy from the light collecting system to the reaction center (Stirbet & Govindjee, 2011; Schansker *et al.,* 2014). The lower energy loss of control plants evidences that, in this condition, there is less energy dissipation in the form of fluorescence, with a consequent increase in the formation of ATP and NADPH and assimilation of carbon (Taiz *et*

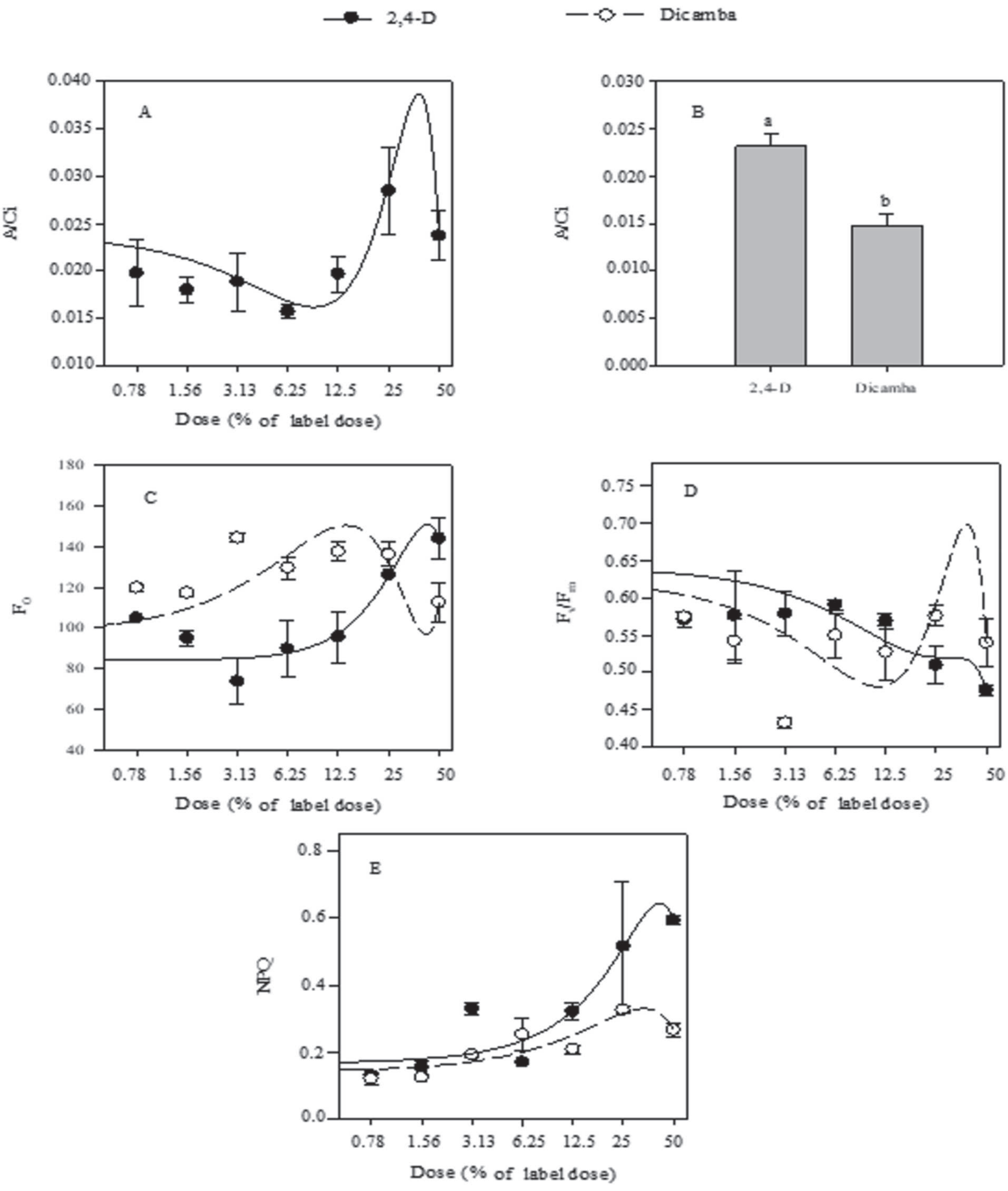

Figure 3: Rubisco carboxylation efficiency (A/Ci) (A and B), fluorescence (F_0) (C), maximum quantum yield (F_v/F_m) (D) and non-photochemical quenching (NPQ) (E) in Brazilian Peppertree plants submitted to doses of 2,4-D and Dicamba at 45 DAA after applying the treatments.

al., 2017). The increase in F_0 is mainly associated with a reduction in the electron receptors of plastoquinone, which results in a delay of the electron transfer chain in the PSII and the light uptake of the protein separation complexes Chl *a*/*b* in the PSII (Baker, 2008; Ashraf & Harris, 2013; Hazrati *et al.*, 2016).

The highest values for F_v/F_m of PSII observed in plants without herbicide drift (Figure 3D) indicate that most of the light energy is directed to the photochemical step of photosynthesis instead of lost by chlorophyll *a* fluorescence (Baker, 2008; Berghetti *et al.*, 2019). Moreover, the lower Fv/Fm observed in plants at higher doses may be related to the reduction of chlorophyll content in leaves (Cambrollé *et al.*, 2015), due to the damage caused by herbicides to chloroplasts, membranes and the plant vascular system (Grossman, 2010). The reduction in Fv/Fm characterizes a lower amount of energy absorbed by the plant through the complex antenna, and it is used to transport electrons and produce dry matter (Tiecher *et al.*, 2016, Tiecher *et al.*, 2017).

The increase in NPQ observed in plants subjected to the two highest doses of herbicides is related to a reduction in the values of F_v/F_m, indicating that the plants are dissipating energy (Hazrati *et al.*, 2016) as heat probably due to damage to the photosynthetic apparatus caused by the destruction of chloroplasts. This is because the electron transfer chain becomes saturated and the proton accumulation increases, increasing the NPQ (Lambrev *et al.*, 2012; Porcar-Castell *et al.*, 2014). The higher NPQ value indicates the ability to mitigate the negative effects of stress at the chloroplast level since these organelles have the ability to dissipate excess excitation energy (Li *et al.*, 2014, Ismail *et al.*, 2014).

The results of the present study indicate that the increase in doses of the herbicides 2,4-D and Dicamba caused a greater phytotoxicity due to drifts to Brazilian peppertree plants as they promoted negative changes in the photosynthetic activity of plants. This is an important factor to be considered since the potential increase in the use of these herbicides may have a negative impact on the development of plants in drift events. Dicamba presents a greater potential of damage to the species.

4. CONCLUSIONS

The increase in the doses of the herbicides 2,4-D and Dicamba causes a greater phytotoxicity to Brazilian peppertree plants. Dicamba caused a greater phytotoxic and photosynthetic damage, the Brazilian peppertree plants presented less net assimilation rate of CO_2, stomatal conductance, intercellular CO_2 concentration, transpiration rate, Rubisco carboxylation efficiency and also affected the fluorescence emission of chlorophyll *a* when compared to 2,4-D.

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