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Phytotoxicity in soybean crop caused by simulated dicamba drift

Abstract – The objective of this work was to evaluate injury symptoms on soybean not tolerant to dicamba (3,6-dichloro-2-methoxybenzoic acid), as well as crop yield, after the application of sub-rates of the herbicide to simulate physical drift in tropical conditions. Dicamba rates of 0, 5.8, 14.4, 28.8, 57.6, and 576 g acid equivalent per hectare were applied at the vegetative (V3) and reproductive (R1) stages of soybean, using a backpack sprayer pressurized with CO_2 , equipped with air-induction flat fan spray nozzles; the pressure and rate of application were 250 kPa and 200 L ha⁻¹, respectively. Visible injury, the soilplant analysis development (SPAD) index (leaf chlorophyll content) at 14 days after herbicide application, and soybean crop yield were evaluated. These variables were influenced by the crop stage in which the dicamba rates were applied. Rates below 28.8 g ha⁻¹ caused less injury to soybean when applied at the R1 stage; however, there were no differences in yield between stages. A 1% dicamba drift in tropical conditions reduces soybean yield by 12%.

Index terms: *Glycine max*, phenological stage, symptomology, synthetic auxin, yield.

Fitotoxicidade na cultura de soja causada por deriva simulada de dicamba

Resumo – O objetivo deste trabalho foi avaliar os sintomas de injúria em soja não tolerante ao dicamba (ácido 3,6-dicloro-2-metoxibenzóico), bem como a produtividade da cultura, após a aplicação de subdoses do herbicida para simulação da deriva física em condições tropicais. Doses de dicamba de 0, 5,8, 14,4, 28,8, 57,6 e 576 g de equivalente ácido por hectare foram aplicadas nos estádios vegetativo (V3) e reprodutivo (R1) da soja, com uso de pulverizador costal acionado por CO₂, dotado de pontas de jato plano com indução de ar; a pressão e a taxa de aplicação foram de 250 kPa e 200 L ha⁻¹, respectivamente. Foram avaliados injúria visual, índice "soil-plant analysis development" (SPAD) (teor de clorofila das folhas) aos 14 dias após a aplicação do herbicida e produtividade da cultura de soja. Essas variáveis foram influenciadas pelo estádio da cultura em que as doses de dicamba foram aplicadas. Doses inferiores a 28,8 g ha⁻¹ causaram menor injúria na soja quando aplicadas no estádio R1; contudo, não houve diferenças de produtividade entre os estádios. A deriva de dicamba de 1%, em condições tropicais, reduz a produtividade da soja em 12%.

Termos para indexação: *Glycine max*, estádio fenológico, sintomatologia, auxina sintética, produtividade.

Introduction

Brazil expressively participates in global food production, and soybean [*Glycine max* (L.) Merr.] is the main crop in the country:



during the 2019–2020 harvest, 121 million tons were produced on the 36.9 million hectares cultivated (Acompanhamento..., 2020). This production represents 27% of the total soybean produced worldwide (FAO, 2018).

To achieve profitable yields, pests and diseases that attack soybean crops need to be controlled, as do the weeds that compete for light, water, and nutrients. The application of herbicides is the primary method of weed control (Oliveira et al., 2019). However, the inappropriate use of these products has led to the emergence of many cases of weed resistance (Heap, 2019), especially after the development of glyphosatetolerant soybean cultivars. As an alternative for controlling glyphosate-resistant weeds, soybean cultivars tolerant to dicamba (3,6-dichloro-2methoxybenzoic acid), a broad-spectrum herbicide, were developed and commercially introduced into the United States market in 2016 (Wechsler et al., 2019). In 2018, dicamba-tolerant cultivars represented 43% of the total soybean area cultivated in the country (Wechsler et al., 2019).

One of the consequences of the increased number of dicamba applications on tolerant soybean and cotton (Gossypium spp.) cultivars in the United States was the appearance of phytotoxic symptoms in herbicidesusceptible crops located close to the area receiving the herbicide (Zaccaro et al., 2020). It is estimated that 1.4 million hectares of soybean cultivated in 2018 were damaged by physical drift, volatility, and spray tank contamination due to dicamba applications (Wechsler et al., 2019), as non-tolerant soybean is very susceptible to this herbicide (Johnson et al., 2012). Solomom & Bradley (2014) reported that 0.03 g ha⁻¹ dicamba, equivalent to 0.005% of the used rate, is sufficient to cause visible symptoms of injury on soybean plants. However, visible injury is not an adequate parameter to estimate losses in soybean yield because it is a subjective and highly variable characteristic (Foster et al., 2019).

Soybean damage from dicamba drift is related to soil and climate conditions before, during, and after exposure to the herbicide (Griffin et al., 2013). Besides directly affecting the drift process (Hilz & Vermeer, 2013; Holterman et al., 2017), temperature and relative humidity also affect the physiological response of the plant (Egan et al., 2014). The rainfall regime is another factor that influences the injury caused by dicamba and the reduction of crop yield (Foster & Griffin, 2018). Moreover, the sensitivity of soybean to dicamba depends on the phenological stage of the crop at the time of exposure. Studies have shown that the yield losses of soybean exposed to dicamba at the reproductive stage were higher than at the vegetative stage (Johnson et al., 2012; Griffin et al., 2013; Soltani et al., 2016; Kniss, 2018). The effects of dicamba subrates on susceptible crops have also been reported (Kruger et al., 2012; Colquhoun et al., 2014; Egan et al., 2014; Dittmar et al., 2016; Hatterman-Valenti et al., 2017; Kniss, 2018; Jones et al., 2019a, 2019b; Zhang et al., 2019). However, there is a lack of information about tropical conditions, especially in Brazil, which shows the need to obtain regionalized data to establish better technologies for applying the product, in order to avoid the undesired effects of drift and/or tank contamination.

The objective of this work was to evaluate injury symptoms on soybean not tolerant to dicamba (3,6-dichloro-2-methoxybenzoic acid), as well as crop yield, after the application of sub-rates of the herbicide to simulate physical drift in tropical conditions.

Materials and Methods

The study was carried out at the Capim Branco experimental farm (19°08'40"S, 47°57'23"W, at 838 m altitude) of Universidade Federal de Uberlândia, located in the municipality of Uberlândia, in the state of Minas Gerais, Brazil. According to Köppen's classification, the climate of the region is of the Aw type, humid tropical, with a rainy summer (October to March) and dry winter (April to September).

The experiment was conducted in a randomized complete block design, in a 6×2 factorial arrangement, with four replicates. The first factor corresponded to six dicamba rates and the second, to the soybean phenological stage during application, i.e., vegetative (V3) or reproductive (R1). The experiment was repeated in two different farm areas during the 2017 harvest, separated from each other by 100 m. In both experimental areas, the same crop treatments – such as fertilization and pest, disease, and weed control – were applied according to crop requirements (Tecnologias..., 2013).

The dicamba rates used were 0, 5.8, 14.4, 28.8, 57.6, and 576 g acid equivalent (ae) per hectare,

corresponding to 0, 1.0, 2.5, 5.0, 10, and 100% of the 1.2 L ha⁻¹ rate of the herbicide Banvel (Syngenta Proteção de Cultivos Ltda., São Paulo, SP, Brazil) with a concentration of 480 g ae L⁻¹. The rates between 5.8 and 57.6 g ae ha⁻¹ were applied to simulate the drift effect. The 0 and 576 g ae ha⁻¹ rates were used as a standard in the evaluations of the plots that did not receive the herbicide and of those that received the full rate (100%), respectively. The Nidera 6906 RR2 IPRO cultivar, tolerant to glyphosate but susceptible to dicamba, was used. Seeds were mechanically sown on 12/9/2016, in a 0.45 m interrow spacing, to obtain a final population of 400 thousand plants per hectare. The experimental plot was composed of five lines, each 5 m in length, and the useful plot was composed of the three central lines, each 3 m in length; the useful area was of 4.05 m².

The herbicide was applied using the Pulverizador Pesquisa backpack sprayer with a constant CO₂ pressure (Herbicat, Catanduva, SP, Brazil), equipped with a boom with six AIXR 110015 spray nozzles (Spraving Systems Co., Glendale Heights, IL, USA) spaced 0.5 m apart and kept 0.5 m above the crop canopy. Application rate, pressure, and operating speed were 200 L ha⁻¹, 250 kPa, and 1.2 m s⁻¹, respectively. During the treatments with dicamba, the plot receiving the herbicide was bounded by a physical barrier (plastic tarp) positioned vertically to prevent drops from reaching neighboring plots. Before each treatment was applied, the hydraulic circuit of the sprayer was cleaned. The weather conditions recorded at the beginning and end of the treatments were as follows: temperature of 21-25°C, relative humidity of 84–70%, and wind speed of 0.8–1.4 m s⁻¹, respectively.

The estimate of visible injury, the soil-plant analysis development (SPAD) index (leaf chlorophyll content) at 14 days after herbicide application (DAA), and crop yield were evaluated. Herbicide injury to plants was based on visual scores, ranging from 0% (absence of symptoms) to 100% (complete plant death), according to the scale proposed by Robinson et al. (2013). The SPAD index was obtained by the SPAD-502 chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan), in the third completely expanded trifoliate leaf of three plants from each useful plot. This index was recently used by Wells et al. (2019) to evaluate the injury promoted by dicamba in pecan [*Carya illinoinensis* (Wangenh.) K.Koch] plants in the state of Georgia, United States.

Yield was determined by manual harvesting, sorting, separation of impurities, and weighing of grains. The humidity of the samples was adjusted to 13%, and mass was extrapolated to kg ha⁻¹.

The data of visible injury, the SPAD index, and vield were subjected to assumptions of normality of residuals, homogeneity of variances, and additivity of blocks, respectively, checked by Shapiro-Wilk's, Levene's, and Tukey's tests, using the SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA). When data transformation impaired the normal distribution of the residuals or the homogeneity of variances, analyses of variance were carried out using the original data in the Sisvar statistical software, version 5.6 (Ferreira, 2011). The data from both experimental areas were combined since the ratio between the largest and the smallest mean squared residues (MSR) for each variable was < 3 (Gomes & Guimarães, 1958). For each herbicide rate, comparisons between phenological stages were made by Tukey's test, at 5% probability. Rate-response herbicide regressions were adjusted by the loglogistic four-parameter model using the drc package of the R software, version 3.2.3 (R Core Team, 2015), according to the following equation (Ritz et al., 2015):

$$y = c + \{d - c/1 + exp [b (log x - log e)]\}$$

where y is the response variable; b is the slope at the inflection point; c and d are the lower and upper limits, respectively; e is the inflection point; and x is the herbicide rate. The lower limit (c) of the model was set to 0 due to a better fit to the data.

Results and Discussion

There was a significant interaction between herbicide rate and soybean phenological stage for all characteristics evaluated (Table 1). The application of 5.8, 28.8, and 57.6 g ae ha⁻¹ at the R1 stage resulted in an average estimate of visible injury 16% lower than that at the V3 stage at 14 DAA. Conversely, applying 576 g ae ha⁻¹ dicamba caused greater injury to soybean at R1 (97%) than at V3 (92%), whereas the rates of 0 and 14.4 g ae ha⁻¹ produced similar injury levels of 0 and 41%, respectively. The absence of phytotoxic symptoms at 14 DAA in the plants that were not treated suggests there was no cross-contamination of plots during and after herbicide applications. Although the rate of 576 g ae ha⁻¹ did not cause complete plant death at 14 DAA, it was not possible to detect leaf chlorophyll content, and, therefore, the SPAD index was considered null, both in plants that received herbicide application at V3 and R1 (Table 1). For the remaining rates, the leaves of the plants at the R1 stage had a lower chlorophyll content than those at the V3 stage. These results show that using the SPAD index to evaluate the phytotoxicity caused by dicamba in soybean plants was not adequate since varying values were obtained even when the plants did not receive the herbicide. Therefore, it is necessary to identify other forms of physiological evaluation based on leaf chlorophyll content to better characterize the phytotoxicity caused by dicamba.

The plants that received rates from 0 to 28.8 g ae ha⁻¹ dicamba had similar yields at both stages; however, with the 57.6 g ae ha⁻¹ rate, soybean produced 1,035 kg ha⁻¹ more at the V3 stage than at the R1 stage, whose yield was zero (Table 1). These findings indicate that the differences in the estimate of visible injury and SPAD index between the two phenological stages did not lead to yield differences. When 1.0, 2.5, and 5.0% of the full rate (576 g ha⁻¹) were used, the average yield was reduced by 12, 18, and 25%, respectively, compared with the control treatment, which had an average yield of 3,568 kg ha⁻¹. Therefore, drift reduction methods, such as safety strips, nozzles with

air inclusion, adjuvants, air assistance, and protected bars in the sprayers, which provide dicamba drift of less than 1%, can be used to reduce the damage in susceptible crops and in those adjacent to the treated area.

The log-logistic four-parameter model did not fit the SPAD index data. As expected, the percentage of plant injury increased with the increase of the herbicide rate, although in a nonlinear manner (Figure 1 and Table 2). Up to the rate of 57.6 g as ha^{-1} , the rate of increase of the observed injury was similar for both phenological stages of soybean. From this rate on, there was a higher rate of increase of the injury at the R1 stage. In addition, a sharper reduction in sovbean yield was observed when plants were exposed to rates of dicamba above 28.8 g ha⁻¹, also at the R1 stage. The rates needed to cause zero yield were estimated at 57.6 and 200 g ae ha⁻¹ when applied at the R1 and V3 stages, respectively. With these results, it is possible to infer that the risk of damage to soybean exposed to dicamba drift will be higher, between 5 and 35%, at the R1 stage. To reduce these risks, the United States Environmental Protection Agency (EPA, 2019) has proposed the application of dicamba on herbicide-tolerant soybean before the plants reach the V4 stage or up to 45 days after the sowing date of the crop. As soybean sowing is concentrated in an established period for each production region, this recommendation reduces the

Table 1. Estimate of visible injury, soil-plant analysis development (SPAD) index (leaf chlorophyll content) at 14 days after herbicide application, and yield obtained for the Nidera 6906 RR2 IPRO soybean (*Glycine max*) cultivar as a function of dicamba rates (acid equivalent) at two phenological stages of the crop⁽¹⁾.

Phenological	Rate (g ae ha ⁻¹)						
stage ⁽²⁾	0.0	5.8	14.4	28.8	57.6	576.0	
			Visible in	njury (%)			
V3	0a	38a	41a	52a	64a	92b	
R1	0a	32b	40a	44b	54b	97a	
	$F_{\text{rate x stage}}^{(3)} = 30.6^*; \text{ MSD} = 2.0; \text{ CV} (\%) = 4.5$						
	SPAD index						
V3	36a	38a	43a	41a	40a	0a	
R1	25b	34b	34b	31b	29b	0a	
	$F_{\text{rate x stage}} = 8.3^*; \text{ MSD} = 3.0; \text{ CV } (\%) = 10.8$						
	Yield (kg ha ⁻¹)						
V3	3,518a	3,192a	3,011a	2,670a	1,035a	0a	
R1	3,619a	3,107a	2,850a	2,693a	0b	0a	
]	$F_{rate x stage} = 73.4*; MSD$	= 553.0; CV (%) = 26.	2		

⁽¹⁾Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. ⁽²⁾V3, vegetative; and R1, reproductive. ⁽³⁾F-value calculated for the interaction between dicamba rate and soybean phenological stage. MSD, minimum significant difference. *Significant at 5% probability.

Andersen et al. (2004) evaluated the yield of two soybean cultivars (PB1901RR and AG1301RR) exposed to dicamba rates at the V3 stage in North America and observed that the rate equivalent to 1% of the full rate caused a 14% reduction in yield, which is close to the value of 12% obtained in the present study, even though the experiments were carried out in different geographic regions. Kniss (2018) conducted a meta-analysis of 11 published field studies on soybean response to dicamba and concluded that cultivation at the reproductive stage was two to six times more susceptible to the applied herbicide than at the vegetative stage. The results found by the author differ partially from those of the present work since, here, soybean had a similar sensitivity to dicamba when exposed to a rate of 28.8 g ae ha⁻¹, either at the V3 or R1 stage. These differences can be attributed to the distinct tolerance of cultivars, size of the used droplets, application rate, meteorological conditions during application, and volatility of the product, despite not being the focus of the present study.

The obtained findings can be useful to understand both the effect of dicamba drift on non-tolerant soybean crops due to treated neighboring areas or the occurrence of injuries promoted by the detachment of residues of this herbicide in the carrier volume in subsequent applications, after an incorrect cleaning of the hydraulic circuit of the sprayer. Therefore, exposure to dicamba sub-rates interferes with plant development and should be avoided.



Figure 1. Rate-response curves of the estimate of visible injury 14 days after herbicide application (A) and yield (B) obtained for the Nidera 6906 RR2 IPRO soybean (*Glycine max*) cultivar as a function of the application of dicamba rates (acid equivalent) at two phenological stages of the crop. V3, vegetative stage; and R1, reproductive stage.

Table 2. Parameters of the log-logistic four-parameter model used to estimate visible injury on soybean (*Glycine max*), as well as crop yield, after exposure to dicamba at two phenological stages of the crop.

Variable	Phenological stage ⁽¹⁾	Parameter ⁽²⁾				
	-	b (±SE ⁽³⁾)	d (±SE)	e (±SE)		
Injury	V3	-0.34 (0.04)	161.61 (25.13)	245.98 (235.83)		
	R1	-0.32 (0.01)	307.42 (30.68)	6,675.73 (3,424.36)		
Yield	V3	3.18 (1.13)	3,269.65 (182.97)	45.64 (4.83)		
	R1	10.50 (19.97)	3,203.47 (120.75)	33.74 (10.15)		

⁽¹⁾V3, vegetative; and R1, reproductive. ⁽²⁾b, slope at inflection point; d, upper limit; and e, inflection point of the log-logistic model. ⁽³⁾Standard error.

Conclusions

1. Sub-rates of the dicamba herbicide promote injuries on non-tolerant soybean (*Glycine max*) plants, as well as yield reduction, which can reach 12% when 1% of the full rate is applied.

2. The effect of dicamba sub-rates on soybean health and yield depends on the phenological stage of the plant at the time of exposure.

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