




Drift of 2,4-D and dicamba applied to soybean at vegetative and reproductive growth stage

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ABSTRACT: *The introduction of dicamba and 2,4-D-resistant soybean will increase the use of auxin herbicides for management of herbicide-resistant weeds, increasing risk of drift in non-target crops. The field experiment was conducted in 2016/17 to evaluate injury, growth, yield, germination and seeds vigor of soybean effects to simulated drift of 2,4-D and dicamba applied to soybean at the V₃ and R₂ growth stages. To simulate drift, 2,4-D was applied at 0; 5.16; 10.4; 20.8 e 41.5g ae ha⁻¹ and dicamba at: 0; 3.7; 7.4; 14.9 e 29.8g ae ha⁻¹. The injury of the dicamba is greater than 2,4-D, and the V₃ stage is more susceptible to both herbicides. The greatest reductions in soybean yield follow the drift of dicamba in R₂ and 2,4-D in V₃. The physiological quality seeds of the soybean is reduced by dicamba and 2,4-D drift at both the V₃ and R₂ stages of the soybean. Soybean is highly sensitive to low rates of 2,4-D and dicamba at the vegetative or reproductive growth stages. Dicamba causes greater negative effects than 2,4-D on soybean. The low rate of 2,4-D and dicamba reduce germination and vigor seed on soybean offspring.*

Key words: dose response, injury, germination, vigor, synthetic auxin.

Deriva de 2,4-D e dicamba aplicado em estágio vegetativo e reprodutivo da soja

RESUMO: *A introdução de cultivares resistentes ao dicamba e 2,4-D aumentará o uso destes herbicidas para o manejo de ervas daninhas resistentes a herbicidas, aumentando o risco de deriva em culturas não-alvo. Assim, o objetivo do trabalho foi avaliar a fitotoxicidade, crescimento, produtividade, germinação e vigor de sementes de soja exposta deriva simulada de 2,4-D e dicamba nos estágios vegetativo e reprodutivo da soja. O experimento foi conduzido a campo na safra 2016/17. Para simular a deriva, 2,4-D foi aplicado nas doses de 0; 5,16; 10,4; 20,8 e 41,5g e.a. ha⁻¹ e dicamba nas doses de 0; 3,7; 7,4; 14,9 e 29,8g e.a. ha⁻¹. A fitotoxicidade de dicamba é maior que 2,4-D, sendo o estágio V₃ mais suscetível para ambos herbicidas. As maiores reduções na produtividade da soja sucedem a deriva de dicamba em R₂ e 2,4-D em V₃. A qualidade fisiológica das sementes de soja é reduzida em função da deriva de dicamba e 2,4-D em aplicação nos ambos estágios V₃ e R₂ da soja. A soja é altamente sensível a baixas concentrações de 2,4-D e dicamba nos estágios vegetativo e reprodutivo. O dicamba causa maiores efeitos negativos comparado com 2,4-D. Baixas concentrações de 2,4-D e dicamba reduzem germinação e vigor de sementes de soja.*

Palavras-chave: dose resposta, fitotoxicidade, germinação, vigor, herbicidas auxínicos.

INTRODUCTION

Development of soybean cultivars with resistance to synthetic auxins is an important technology and are strategic additional tools to combat herbicide-resistant weeds and others broadleaf weeds problem (SPAUNHORST & SIEFERT-HIGGINS; 2014).

In soybean with resistance to the synthetic auxin herbicides will allow the use of 2,4-D and dicamba in burndown or PRE, and POST soybean without causing crop injuries. However, it is important that this technology can be causing

damage to neighboring crops and native plants by herbicide off-target movement, unintentionally drift or volatilization (OLSZYK et al., 2015). Furthermore, the injury in soybean is increased when some registered post-emergence herbicides are applied followed by synthetic auxin drift (BROWN et al., 2009).

The effects of sub-lethal rate of synthetic auxin in off-target crops are reported to cause substantial injury and yield loss. In soybean; however, there are differences in the damage of these synthetic auxin herbicides and timing exposure, in general, dicamba is more harmful than 2,4-D, especially when

applied during reproductive stages (GRIFFIN et al., 2013; SOLOMON & BRADLEY, 2014). Although, 2,4-D exposure to the V₅ soybean stage influenced more than V₂ and R₂ stage (ROBINSON et al., 2013b). Previous research has documented that synthetic auxins herbicides drift onto sensitive soybean can cause many negative effects, such as reduction of seed weight, lower seed quality, lower seed oil and protein concentration, pod malformation and impacts on progeny (ROBINSON et al., 2013a, 2013b; SOLOMON & BRADLEY, 2014; KESOJU et al., 2016; MILLER & NORSWORTH, 2017).

Synthetic auxin herbicides, at low rate, have similar hormonal properties to natural auxin; nevertheless raising the herbicide rate causes several abnormalities in sensitive broadleaf. Germination process is determined by diverse environmental such as the availability of water, temperature, CO₂, light and hormonal balance. An important hormone, the auxin is involved regulate several hormonal processes, including the interaction with abscisic acid and gibberellic acid, affecting fruit and seed formation (REN & WANG, 2016). In wheat, exogenous application delayed seed germination on the spike (RAMAIIH et al., 2003). Similarly, dicamba applied at reproductive stage of soybean reduces germination, stand, plant height and yield of the offspring soybean (MILLER & NORSWORTH, 2017).

Thus, the objective of this research was to determine the effects of 2,4-D and dicamba simulated drift on soybean injury, height, yield, seed germination and vigor when applied in the vegetative and reproductive stages of growth.

MATERIALS AND METHODS

A field trial was conducted between November of 2016 and April of 2017, at Universidade Federal de Santa Maria, in Frederico Westphalen, RS, Brazil, in the classified soil as typical dystrophic red latosol with clay of 600mg dm⁻³, pH of 6.2 and 3.2% organic matter. During the experiment, the mean temperature were 22.5; 23.0; 24.0; 24.0; 22.1 and 21.2, and the total rainfall was 116; 169; 120; 225; 174 and 180mm, to November, December, January, February, March, and April, respectively.

The field trial was established as a randomized complete block design with four replications. Treatments were 2,4-D at rates of 0, 5.16, 10.4, 20.8 and 41.5g ae ha, and dicamba at 0, 3.7, 7.4, 14.9 and 29.8g ae ha⁻¹ applied at the V₅ and R₂ soybean growth stages to simulate drift. The

soybean was implemented in no-tillage in cover crop (*Avena sativa*) with pre-plant glyphosate application at 50 and 10 days before planting. A glyphosate-resistant soybean cultivar (BMX GARRA IPRO) was planted on November 21, 2016, at 216000 seeds ha⁻¹ and row spacing was 0.45m wide. Each experimental plot contained four rows, measuring 1.8m wide and 4.0m long. The fertilizer was applied at planting at 350kg ha⁻¹ of 05-30-10 NPK. To avoid weed competition effects, the study was conducted under weed-free conditions, where the weed post-emergence consisted of glyphosate (816g ae ha⁻¹) application at 16 and 29 days after emergence. All herbicide treatments were applied with a CO₂-pressurized back-pack sprayer outfitted with 110015 flat-fan nozzles at 100kPa calibrated to deliver 150L ha⁻¹. Herbicide applications were made with a spray boom with four nozzles spaced 50cm apart. The visible injury was not apparent between adjacent treated plots with herbicides.

Visible estimates of soybean injury were collected at four weeks after treatment (WAT), on a 0 to 100% scale relative to untreated, where 0 represented no injury and 100 to complete crop death. In addition, five arbitrarily selected plants for soybean heights, which measured distance from the ground to the tip of the topmost fully expanded leaf. At harvest, the two center rows harvested manually and grain yield was recorded and seed moisture content being adjusted to 13%. Immediately after harvest, samples containing 100g of seeds of each plot were collected and sent to the laboratory for seed germination and vigor analysis. Samples were dried and maintained at 13% moisture and stored at 20 - 25°C for three months.

In the laboratory, germination and vigor seed tests were performed, using the rolled paper towel method, which 100 seeds from each treatment in each replication were distributed on two sheets of germination paper previously humidified with distilled water three times greater than its weight, and then covered with a moistened sheet paper. Rolled sheets of paper were placed in plastic bags and positioned in an upright position. These were left in a regulated germinator to maintain the temperature between 25 and 30°C.

After five days, the test the first count was carried out, the abnormal seedlings were count and removed, and normal and vigorous seedlings, expressed as a percentage, represented seed vigor. Percentage of seed germination was performed adding the values reported for normal seedling in the first and last count, after eight days.

Normality tests were performed using residual data procedure and Shapiro Wilk test. Non transformed means for soybean variables are presented because transformations did not improve the normality of the data. Data were subjected to ANOVA and tested for appropriate interactions. Subsequent linear and nonlinear regression was conducted using Sigmaplot 10.0.

RESULTS AND DISCUSSION

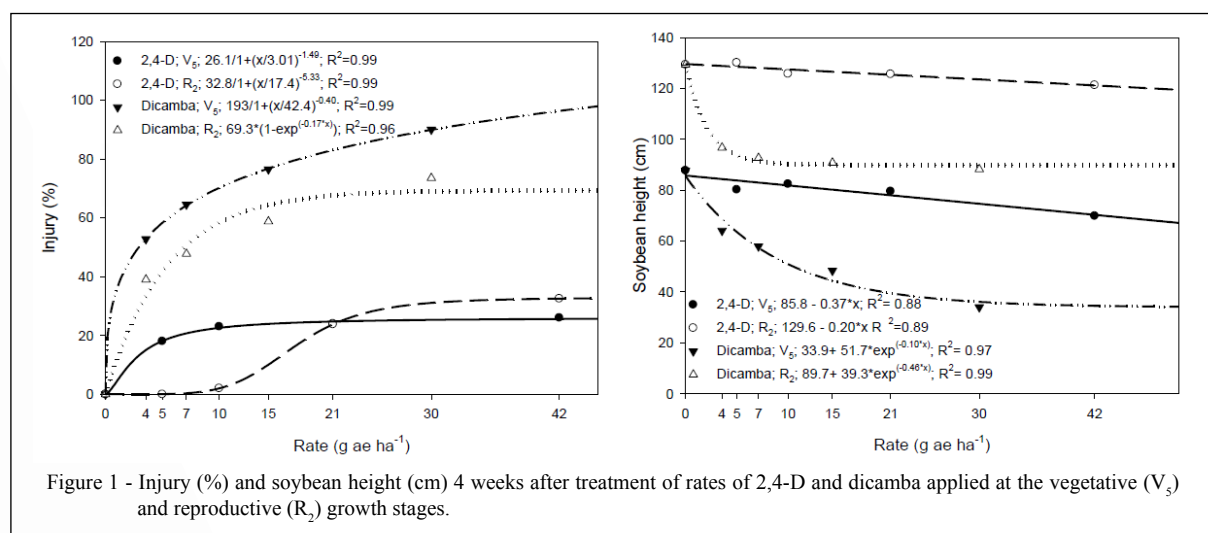
There are interactions effects of herbicide, rate, and stages of growth effects for all variables. Across all herbicide rates and growth stages, the soybean injury increased with increasing herbicide rates, and injury intensity was higher to dicamba than for 2,4-D (Figure 1).

The symptoms appeared differently between herbicides across the growth stages of soybean, where dicamba resulted in higher injuries at the V_5 stage, and the differences on the injury caused by 2,4-D showed to be slight among growth stages at the highest rate (Figure 1). Soybean injury to dicamba exposure had more than doubled compared to 2,4-D, ranging from 35 to 87%, while the 2,4-D injuries ranged from 0 to 31% across both growth stages and herbicide rate. The nonlinear regressions predicted effective rate causing 50% of injury to dicamba applied in the V_5 stage was 3.1g ha^{-1} , while in the R_2 stage was needed a twice rate (7.2g ha^{-1}) (Figure 1). These dicamba rates represented 0.77 and 1.55% of standard herbicide rate of 480g ha^{-1} , respectively. ROBINSON et al. (2013a) reported similar values at 50% of injury, ranging from 1.44 to 4.4g ha^{-1} when

applied in the vegetative stage and from 1.8 to 5.3g ha^{-1} when applied to reproductive stage.

The degree of injury observed in soybean for 2,4-D and dicamba herbicides were similar to those observed in soybean (GRIFFIN et al., 2013; ROBINSON et al., 2013a, 2013b; SOLOMON & BRADLEY, 2014). Soybean exhibited the greatest symptoms injury to 2,4-D between 26 and 32% for V_5 and R_2 stages at high rate, respectively (Figure 1). Conversely, at a lower rate (5.16 and 10.4g ha^{-1}), the 2,4-D displayed no injury when applied in the R_2 stage, but visible soybean injury was noted in V_5 . In contrast, ROBINSON et al. (2013b) reported equivalent soybean injury for 2,4-D at rate ranging from 145 and 245g ha^{-1} . These results are consistent with those reported by GRIFFIN et al. (2013); ROBINSON et al., (2013a, 2013b) and SOLOMON & BRADLEY, 2014, who reported that soybean exposure to auxin herbicides drift causes significant more crop injury when applied during vegetative than reproductive stage.

The highest injury in the vegetative stage of growth for both herbicides is endorsed to higher absorption by young leaf and rapidly growing of early stages plants than late stages of growth (RANA et al., 2014). The analyses of symptoms in younger plants are characterized by disorganization of mesophyll cells, such as changes in shape, an increase in cell volume, a reduction in the number of organelles and cell wall disturbances, which do not occur in older plants (PAZMIÑO et al., 2011). Correlated to this, plants in an early stage of growth have a lower activity of antioxidant enzymes, which would minimize the deleterious effects of



the synthesis of reactive oxygen species caused by herbicides, which would result in damage to proteins and lipids (PAZMIÑO et al., 2011).

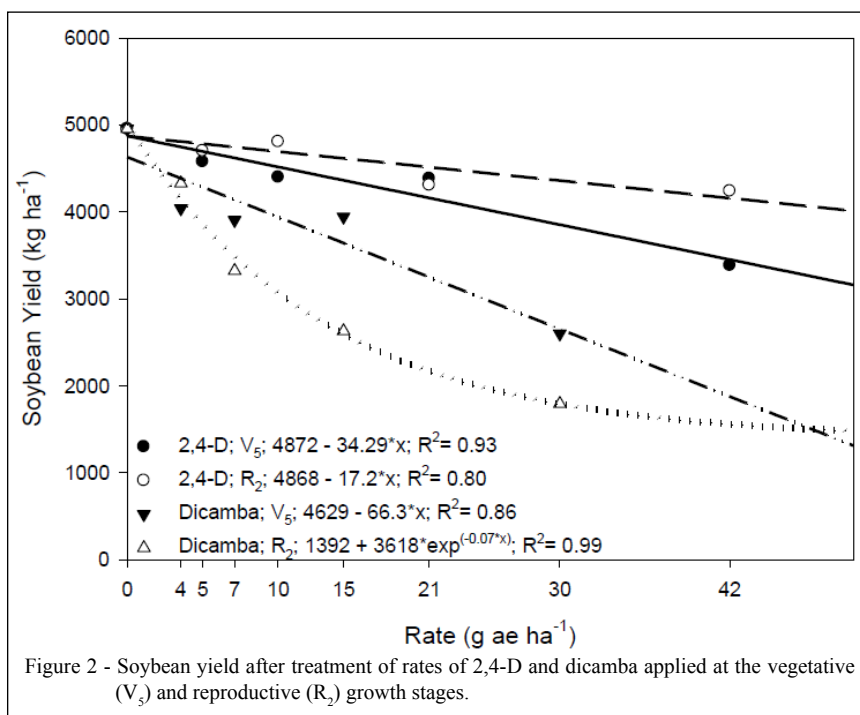
Soybean injury observed had a reflected on plant height and its display more sensitivity to early growth application. For the soybean treated with dicamba at all growth stages showed the highest height reduction after 4 WAT than 2,4-D, being more pronounced in the vegetative than reproductive stage (Figure 1). At the highest dicamba rate, a height reduction of 60% occurred from application at the V_5 stage, while for the 2,4-D showed a linear reduction maximum of 18%. The greater reduction in height due auxin herbicide exposure in V_5 is attributed to the greater injury in early stage compared to late stage, and to the fact that in the R_2 stage the growth in height has already been completed.

Results for soybean height from this study are opposite to those reported by GRIFFIN et al. (2013) that reported greatest soybean height reduction when dicamba was applied at reproductive stage than in vegetative stage, furthermore no reduction in soybean height for 2,4-D at either stage growth. The reduction in plant height may be one of the most important symptoms caused by auxin herbicides that cause yield losses. This lowest height will reduce reduced leaf area, fewer main-stem nodes,

and fewer pods or less seed mass because of reduced photosynthesis (ROBINSON et al., 2013b).

Soybean yield was significantly reduced with increasing auxin herbicides rates, and the yield loss was greater for dicamba than 2,4-D, regardless of growth stage of application (Figure 2). For 2,4-D, the simulated drift rates resulted in less yield in the R_2 growth stage than the V_5 growth stage, for each $g\ ha^{-1}$ of 2,4-D applied at the V_5 stage, soybean yield was reduced $34.3kg\ ha^{-1}$, and at the R_2 stage the yield loss is $17.2kg\ ha^{-1}$. Similarly, soybean yield loss was higher due to drift of 2,4-D at vegetative stage compared to reproductive stage (ROBINSON et al. 2013b). Conversely, the 2,4-D at $28g\ ha^{-1}$ at either application timing did not reduce soybean yield compared to the untreated (SOLOMON & BRADLEY, 2014) at either application timing at $28\ g\ ha^{-1}$ of 2,4-D.

Linear regressions were fit to describe soybean yield loss to dicamba at the V_5 growth stage and 2,4-D to both growth stages, and nonlinear regressions to dicamba at R_2 growth stage (Figure 2). Soybean yield reduction was greatest for dicamba at the R_2 stage, ranging from 16 to 63%. In a similar study, ROBINSON et al. (2013a) reported soybean yield loss of 20% when applied dicamba rates of the 2.38 and $1.17g\ ha^{-1}$ at the V_5 and the R_2 stages, respectively. The estimate of soybean yield loss by dicamba applied at the



R_2 stage is about 800kg ha⁻¹ greater than the V_5 stage, where soybean yield was reduced 64% when dicamba was applied in the R_2 stage, while was observed a 47% yield loss in the V_5 stage.

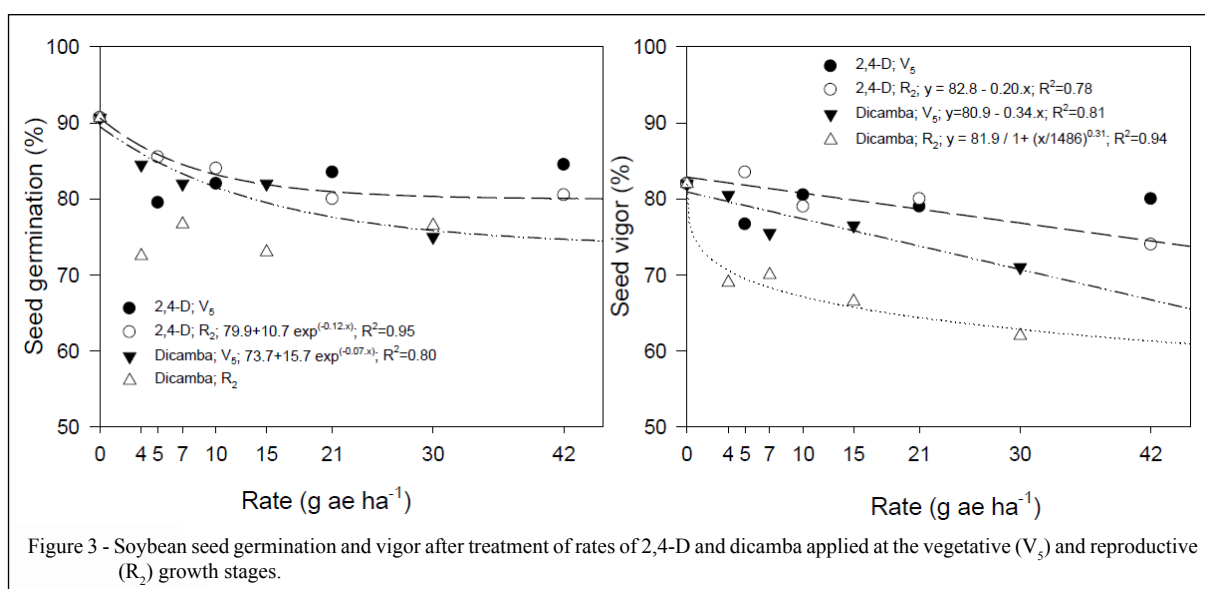
This result is consistent with previous research (GRIFFIN et al., 2013; ROBINSON et al., 2013a; SOLOMON & BRADLEY, 2014), which reported the highest reduction in soybean yield when the plant was exposed to herbicides in late stage compared to early stage. However, dicamba at 28g ha⁻¹ did not result in soybean yield at V_3 application (SOLOMON & BRADLEY (2014).

The dicamba application at the R_2 stage resulted in significant delays in soybean maturity about 15 days in relation to other treatments (no show data). SOLOMON & BRADLEY (2014) also reported 24 days of delay in maturity from 28g ha⁻¹ of dicamba applied during flowering; however, the 2,4-D rates up to 56g ha⁻¹ cause slight or no delay in maturity (KELLEY et al., 2005; SOLOMON & BRADLEY 2014).

Application of auxin herbicides had a negative impact on the germination and vigor of soybean seeds being variable according to the stage of development. A significant exponential response describes seed germination to 2,4-D applied at the R_2 stage and dicamba applied at the V_5 stage and resulted in germination reduction averaged 10 and 14%, respectively, at high herbicide rate (Figure 3). Interestingly, low concentrations of 2,4-D and dicamba applied at the V_5 and R_2 stage, respectively, resulted in lower seed germination than the high herbicide rates.

Similar response also was observed on *Bromus tectorum* with aminopyralid, another auxin herbicide (RINELLA et al., 2013).

These results are consistent with those reported by MILLER & NORSWORTH (2017), which determined negative effects of low rate application of dicamba to soybean in the reproductive stage on seed germination of the offspring, reducing about 60% seed germination. Likewise, application of synthetic auxin herbicide at early reproductive stage reduced the field emergence of the alfalfa (KESOJU et al., 2016). Conversely, NEVES et al., (1998) reporter that 2,4-D at up 10g ha⁻¹ no reduction in soybean seed germination when applied at the R_1 stage; however, later application on reproductive stage reduced the germination. Thereby, it can be inferred that synthetic auxin herbicides cause a reduction in soybean seed quality when are applied at any soybean growth stage. A linear response was observed for soybean vigor seed to effects of 2,4-D applied in the R_2 stage and dicamba at the V_5 stage, while the dicamba at the R_2 stage described logistic response (Figure 3). Seeds from soybean treated with dicamba reduced vigor seed roughly 19% when applied at the R_2 stage and only 8% when applied at the V_5 stage. In addition, at high rate of 2,4-D applied at the R_2 stage reduced vigor seed by 8% compared to untreated. These results are in agreement with those by KESOJU et al. (2016), who observed malformed and abnormal seedling by auxin herbicide applied at early seed development stage in alfalfa, and the effects are more substantially in dicamba than 2,4-D.



The effect of synthetic auxin herbicides on the physiological quality of soybean seeds may be related to changes in hormone levels in the seeds. Since the application of exogenous auxins in soybean seeds negatively regulates the biosynthesis of gibberellic acid, promotes of seed dormancy and reduce seeds germination through seed coat rupture and radicle protrusion delay (SHUAI et al., 2017). These authors argue that auxin regulates seed germination primarily through mediating the abscisic acid signaling pathway, but it was still unclear as to how auxin interacts with the abscisic acid biosynthesis and gibberellin acid biosynthesis/signaling pathways in the seed germination regulatory network. Among other factors that influence germination is low seed oil and protein concentration induced by dicamba application in all growth stages of soybean (ROBINSON et al, 2013b), since the lower seeds oils and proteins contents causes deteriorate during storage, resulting in decreased germination and emergence in the field (WANG et al., 2012; DARGAHI et al., 2014).

Thus, with the commercialization of synthetic auxin resistant soybean, producers, and agronomist should be aware of the recommended spraying techniques to mitigate potential drift, especially focused in adjacent field seed production, since low concentrations of these herbicides will affect the yield and quality of seeds produced. Additional research is needed to understand the impacts of drift of synthetic auxin exposure on others non-target plants as grapevine, citrus orchard, tobacco and native vegetation.

CONCLUSION

Soybean is highly sensitive to low rates of 2,4-D and dicamba at the vegetative or reproductive growth stages. Dicamba causes greater negative effects than 2,4-D on soybean. The highest soybean yield losses follow dicamba exposure in the R₂ stage and 2,4-D in the V₅ stage. The low rate of 2,4-D and dicamba reduced germination and vigor seed on soybean offspring.

CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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