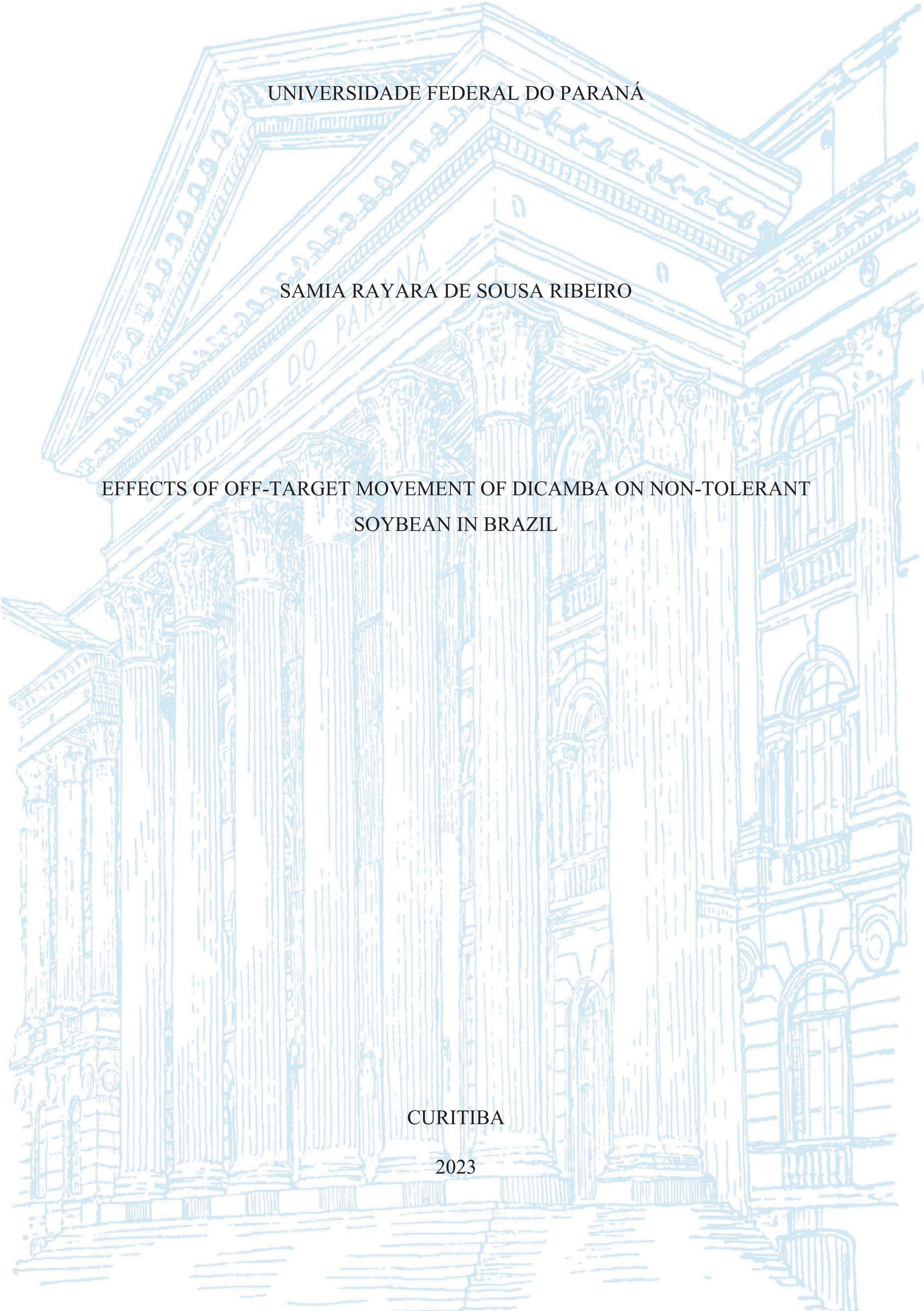

UNIVERSIDADE FEDERAL DO PARANÁ

SAMIA RAYARA DE SOUSA RIBEIRO

EFFECTS OF OFF-TARGET MOVEMENT OF DICAMBA ON NON-TOLERANT SOYBEAN IN BRAZIL

CURITIBA

2023

SAMIA RAYARA DE SOUSA RIBEIRO

EFFECTS OF OFF-TARGET MOVEMENT OF DICAMBA ON NON-TOLERANT SOYBEAN IN BRAZIL

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Agronomia – Produção Vegetal, no Setor de Ciências Agrárias, da Universidade Federal do Paraná, como requisito parcial para obtenção do título de Doutor em Ciências.

Orientador: Prof. Doutor Arthur Arrobas Martins Barroso

Curitiba

2023

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Ribeiro, Samia Rayara de Sousa
Effects of off-target movement of dicamba on non-tolerant soybean in Brazil / Samia Rayara de Sousa Ribeiro. – Curitiba, 2023.
1 recurso online: PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Agronomia (Produção Vegetal).
Orientador: Prof. Dr. Arthur Arrobas Martins Barroso

1. Herbicidas. 2. Dicamba. 3. Soja. I. Barroso, Arthur Arrobas Martins. II. Universidade Federal do Paraná. Programa de Pós-Graduação em Agronomia (Produção Vegetal). III. Título.

Bibliotecária: Telma Terezinha Stresser de Assis CRB-9/944

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO AGRONOMIA
(PRODUÇÃO VEGETAL) - 40001016031P6

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **SAMIA RAYARA DE SOUSA RIBEIRO** intitulada: **EFFECTS OFF-TARGET MOVEMENT OF DICAMBA ON NON-TOLERANT SOYBEAN IN BRAZIL**, sob orientação do Prof. Dr. ARTHUR ARROBAS MARTINS BARROSO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 22 de Novembro de 2022.

Assinatura Eletrônica
20/12/2022 18:57:09.0
ARTHUR ARROBAS MARTINS BARROSO
Presidente da Banca Examinadora

Assinatura Eletrônica
20/12/2022 22:21:18.0
ALFREDO JUNIOR PAIOLA ALBRECHT
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
20/12/2022 17:16:51.0
ALDO MEROTTO JÚNIOR
Avaliador Externo (UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL)

Assinatura Eletrônica
26/12/2022 18:48:25.0
RODRIGO WERLE
Avaliador Externo (UNIVERSITY OF WISCOSIN-MADISON)

ACKNOWLEDGMENTS

I am grateful to the teacher Arthur for this patience and dedication and to Dionisio Gazziero for sharing his knowledge with me. Thanks to the Weed Science Research Group for the support in conducting the research and to the professors and team of UFPR for the partnership. Thanks to my friends for their encouragement during this journey. I am grateful to CAPES for granting the scholarship and to the evaluation board for the precious contributions.

RESUMO

O uso repetitivo e incorreto de herbicidas de um mesmo mecanismo de ação, selecionou espécies de plantas daninhas resistentes à herbicidas. Entre as estratégias adotadas para o manejo destas espécies, está a recuperação do uso de herbicidas antigamente utilizados, como o herbicida dicamba. No Brasil, a possibilidade de uso desse herbicida tem gerado discussões com relação a tecnologia de aplicação deste produto e sua relação com a deriva e volatilização. Neste estudo, no capítulo I, o efeito de baixas doses de deriva de dicamba em soja não-tolerante foi testada para dois estádios de cultivo e para nove cultivares de soja. As aplicações de dicamba foram feitas nos estádios V3 ou R1 de crescimento da soja nos experimentos I e II, e no estádio V3, para nove cultivares no experimento III. Foram realizadas avaliações de fitotoxidade e rendimento de grãos. Em V3, doses abaixo de 7,48 g ae ha^{-1} causaram sintomas de fitotoxidade, e em R1, doses abaixo de 1,87 g ae ha^{-1} causaram injúrias à soja, porém, ambos não comprometeram a produtividade. Quanto às cultivares testadas, a cultivar BRS 388 RR foi mais sensível nas doses de 1,2 e 2,4 g ae ha^{-1} o que pode causar perda de produtividade de até 41%. A escolha das cultivares mais resistentes à deriva de dicamba pode ser uma estratégia para evitar maiores danos de eventuais eventos de deriva. O efeito da volatilização de dicamba também foi testado no capítulo II deste estudo. Sais DGA e BAPMA de dicamba foram aplicados sozinhos e misturados com glyphosate e um agente redutor de volatilidade em soja não-tolerante com a metodologia de low-tunnels. O Experimento I compreendeu 10 tratamentos, incluindo misturas de sais de dicamba DGA com diferentes formulações de glifosato (amônia, dimetilamina, potássio, diamônio) com/sem agente redutor de volatilidade, além do tratamento controle. No Experimento II, seis tratamentos incluíram misturas de sal dicamba DGA com formulação de sal de amônio e potássio de glifosato com/sem agente redutor de volatilidade. O Experimento III incluiu o sal BAPMA dicamba misturado com quatro formulações de glifosato (amónio, dimetilamina, potássio, diamónio) mais tratamento de controle. A fitotoxidade, a altura, número de ramos e peso de grãos por planta foi mensurado. A mistura de dicamba com sal amônio de glyphosate, tanto com sal DGA ou BAPMA de dicamba, não é recomendada, por demonstrar os maiores níveis de lesão e as maiores diminuições da arquitetura das plantas, podendo contribuir na perda de rendimento. A mistura entre dicamba e sal potássico de glyphosate demonstrou ser o menos nocivo às plantas nas condições desse estudo. Para reduzir o risco de volatilização, encoraja-se o uso de um agente redutor de volatilidade em misturas em tanque.

Palavras-chave: movimento fora do alvo, volatilização, deriva de dicamba, soja

ABSTRACT

The repetitive and incorrect use of herbicides of the same mechanism of action, selected herbicides resistant weeds. Among the strategies adopted for managing these species, are the recovery of the use of herbicides formerly used, such as dicamba. In Brazil, the possibility of using this herbicide has generated discussions regarding the technology for applying this product and its relationship with drift and volatilization. In this study, in chapter I, the effect of low doses of dicamba drift on non-tolerant soybean was tested for two growing stages and for nine soybean cultivars. Dicamba applications were made at soybean growth stages V3 or R1 in experiments I and II, and at stage V3, for nine cultivars in experiment III. Evaluations of visual phytotoxicity and grain yield were carried out. In V3, doses below 7.48 g ae ha^{-1} caused phytotoxicity symptoms, and in R1, doses below 1.87 g ae ha^{-1} caused injuries to soybeans, however, both did not compromise productivity. As for the tested cultivars, the BRS 388 RR cultivar was more sensitive at doses of 1.2 and 2.4 g ae ha^{-1}, which can cause a productivity loss of up to 41%. The choice of cultivars that are more resistant to dicamba drift can be a strategy to avoid greater damage from possible drift events. The effect of dicamba volatilization was also tested in Chapter II of this study. DGA and BAPMA salts of dicamba were applied alone and mixed with glyphosate and a volatility reducing agent in soybean non-tolerant with the low-tunnels methodology. Experiment I comprised ten treatments, including mixtures of dicamba DGA salts with different glyphosate formulations (ammonia, dimethylamine, potassium, diammonium) with/without volatility reducing agent, in addition to the control treatment. In Experiment II, six treatments included mixtures of dicamba DGA salt with glyphosate ammonium potassium salt formulation with/without volatility reducing agent. Experiment III included BAPMA dicamba salt mixed with four glyphosate formulations (ammonium, dimethylamine, potassium, diammonium) plus control treatment. Phytotoxicity, height, number of branches and grain weight per plant were measured. The mixture of dicamba with glyphosate ammonium salt, either with DGA or BAPMA salt of dicamba, is not recommended, as it demonstrates the highest levels of injury and the greatest decreases in plant architecture, which may contribute to yield loss. The mixture between dicamba and glyphosate potassium salt proved to be the least harmful to plants under the conditions of this study. To reduce the risk of volatilization, the use of a volatility reducing agent in tank mixes is encouraged.

Keywords: off-target movement, volatilization, drif of dicamba, soybean

List of Ilustrations

Chapter I

Chapter II

Figure 11 - Representation of the low tunnel and the arrangement of the soil boxes inside it.

Table list

Chapter I

Chapter II

Summary

INTRODUCTION

Weeds began to stand out when they began to interfere in human activities, growing spontaneously in agricultural soils and/or in other areas of interest, especially where unwanted, and causing economic damage by weed competition. It is estimated that 20% of the total food produced in the world is lost due to the presence of weeds, which cause losses in the order of 28 billion dollars per year to countries like the United States and Canada (Oerke, 2006; Soltani et al., 2016).

In the 40's, the control of these plants started with the use of organic molecules, called herbicides. In the middle of World War II, the discovery of the 2,4-D herbicide (dichlorophenoxyacetic acid) and its selective control action only for broad leaves leveraged the use of these products. In the following years, companies dedicated themselves to synthesizing new molecules with pesticide actions, until the discovery of the herbicide glyphosate (Peterson, 2016).

In a second phase in weed control, transgenic crops with resistance to herbicides emerged. In 1996, the cultivation of glyphosate tolerant soybean, RR soybean, was released. As a result, the herbicide glyphosate passed to be no longer applied only in crop burndown and but also in post-emergence of plants. The use of different herbicides and the use of herbicides in pre-emergence are then reduced, with weed management undergoing major transformation (James, 2012; Norsworthy et al., 2012).

However, the ease that RR cultures brought, based on a unique and repetitive control tool, also brought problems. The introduction of herbicide tolerant crops was promoted as a way to simplify weed management and increase weed control. However, the possibility of selecting glyphosate-resistant weeds was not considered (Parochetti, 1978). With the high control efficiency of the product, added to the extent of its use, adapted species prevailed in crops (Benbrook, 2016).

Such events led companies in the sector to look for alternatives in the control of weeds, since it has been more than 20 years since a new mechanism of action has been discovered (Duke, 2012). Among these strategies, in addition to the development of new cultivars of cultivated species with tolerance to herbicides, is the recovery of other herbicides previously used, such as the dicamba herbicide, which had its first record of use in 1967 (EPA, 2006).

The dicamba herbicide is an option for controlling broadleaf weeds, especially those resistant to the glyphosate herbicide, such as plants of the *Conyza* genus (Tan et al., 2005). Recent developments of genetically modified soybean and cotton varieties with tolerance to growth-regulating herbicides, including dicamba, will allow this compound to be used with greater flexibility. However, this new technology can expose susceptible crops to airborne herbicide transport, reaching non-target organisms (Egan et al., 2014).

This technology cannot be used in the same way as the RR technology was used. In addition to the problem of the probability of selection of resistant weeds, as already occurs in other countries such as *Amaranthus hybridus*, *Chenopodium album*, among others (Heap, 2018), there are some precautions with this technology related to product application and positioning technology. The drift process can be defined as the movement of droplets applied by the wind, preventing them from reaching the target, or reaching unwanted targets (Silva, 1999). On the other hand, volatilization (transformation of the product into steam) is likely to occur due to the vapor pressure of the product (4.5 mPa at 25°C), being a herbicide with moderate volatility (Bunch; Gervais, 2012), which causes the product to evaporate from the applied surface and is susceptible to drift.

Potential losses related to air transport of dicamba have been reported in several productive fields over the years. Therefore, studies are needed, especially with regard to product application technology and its relationship with drift/volatilization under Brazilian conditions. The hypothesis of this work is that ultra-low concentrations of dicamba from product drift and volatilization cause significant visual damage that can lead to loss of productivity. It is important to investigate what happens both for the effectiveness of the use of the product and drift when dicamba is applied into tank mixes with glyphosate and other products, which will be the trend for years to come. In this sense, the greater the information generated, especially in field conditions, weeds and national cultivars, the greater the food and technical security promoted by the future use of dicamba in Brazil. The objective of this study was therefore to analyze the damage caused by the drift and volatilization of dicamba in non-tolerant soybeans, including when mixed in a tank with other products.

Chapter I

SIMULATED DRIFT OF DICAMBA IN SOYBEAN CULTIVARS

ABSTRACT

To provide new information of dicamba use and possible effects in Brazil, three experiments were carried out simulating herbicide drift on non-tolerant soybeans. Dicamba applications were made in V3 or R1 soybean growth stages in experiments I and II, and in V3 stage, for nine cultivars in experiment III. Visual injury and soybean grain yield assessments were performed. Experiment I indicated that in V3, dicamba concentrations below 7.48 g ae ha^{-1} caused injury symptoms, but did not influence soybean productivity, the same occurring for concentrations below 1.87 g ae ha^{-1} in R1. In experiments II and III, in V3, despite causing severe injury, dicamba did not reduce soybean yield, same as for concentrations below 4.8 g ae ha^{-1} in R1. The sensitivity of soybean to dicamba was higher in the cultivar BRS 388 RR at concentrations of 1.2 and 2.4 g ae ha^{-1}, which may cause loss of productivity of up to 41%. The cultivar BRS 511 would be more suitable when exposed in an eventual dicamba drift, as it showed less sensitivity to the herbicide. Dicamba can cause irreversible damage in the cultivation of non-tolerant soybeans, with mayor problems occurring when plants were exposed to drift in reproductive stage of growth. The choice of cultivars is fundamental previewing less drifts problems in field.

Keywords: auxin herbicide, off-target movement, dicamba injury, reproductive stage of development drift, non-tolerant crops

INTRODUCTION

By introducing genetically modified (GM) herbicide-resistant crops, weed management has changed, e.g., with glyphosate, which is now used not only for burdown purposes, but also in post-emergent crops applications (Mueller; Steckel, 2019). Hence, the use of different herbicide molecules and mechanisms of action has decreased, enhancing herbicide selection pressure on weeds (Norsworthy et al., 2012). Due to an increase in reported cases of resistant weeds trough last decade, there is a demand to develop new herbicide-resistant crops technologies (Heap, 2020; Kruger et al., 2010).

Among different alternatives, auxinic herbicide-tolerant soybean has become a viable option to control broadleaf weeds in cropping systems using old herbicides such 2,4-D or dicamba (Behrens et al., 2007; Byker et al., 2013; Cahoon et al., 2015; Silva et al., 2018). Dicamba is a synthetic auxin herbicide, which stimulates cellular elongation and differentiation. However, high concentrations of these hormones, included even in low herbicides concentrations, leads to abnormal and uncontrollable plant and cellular growth, foliar and stem epinasty, leaf chlorosis, damage of cell membranes, damage in vacuoles and vascular cell system, resulting in tissue necrosis and plant death (Bunch et al., 2012).

In a transcriptomics approach to investigating the mode of auxin herbicides, McCauley et al. (2020) found that synthetic auxinic herbicides regulate a set of genes related to photosynthetic processes. This action on the photosynthetic process may be the main factor behind the death of plants treated with auxin herbicides. Auxin herbicides consistently regulate ABA biosynthesis and downregulation of photosynthesis-related genes in response to auxin herbicides may be due to the action of auxin and ABA (McCauley et al., 2020).

Nevertheless, dicamba application may expose non-tolerant crops to damage, due to the non-target herbicide movement, majority caused by drift. The movement of pesticides away from the target can involve several factors, such as spray equipment, wind speed, droplet size and nozzle type, sprayer pressure, weather conditions and properties of the herbicide formulation (Bales; Sprague, 2020). From spray application, particle drift may vary from one to 16% of the total sprayed volume (Maybank et al., 1978; Wolf et al., 1992). Studies indicate that the drift particles of an area sprayed with dicamba can occur from 1.1 and 1.5%, at 2.2 m s^{-1} and 3.6 m s^{-1} wind speed, respectively, even with air induction nozzles, AIXR (Air Induction Xtend Range) (Alves et al., 2017). These drifts, as little as 0.03 g ae ha^{-1}, may injure soybean crop (Kniss, 2018). Dicamba symptoms can be easily detected in field, even at low concentrations and there is concern about their effects on crop yield (Foster et al., 2019).

When investigating the extent of dicamba injury in non-tolerant crops, several state departments of agriculture in the United States reported injuries. In 2017, 2,708 injury cases were reported and in 2018, there were 600 cases, in crops such as soybeans and cotton (Foster, 2019). Researchers estimated that, only in the United States, approximately 445,000 thousand hectares of non-tolerant soybean were injured by off-target movement of dicamba (Bradley, 2017; 2018). In Brazil, there is no previous studies related with dicamba injuries on different soybean cultivars and there are several differences in the soybean cultivation among countries. In Brazil, besides climate differences, the commercial soybean cultivars presents indeterminate growth habit, that is, continues to growth (new nodes in the stem) even after soybean anthesis, what can severely change herbicide injuries and plant recovery after exposure.

Due to the reports of injury of dicamba in soybean, research approaching off-target movement of this herbicide have been carried out aiming to identify the effects of dicamba, as well as dicamba combined with other products, on non-tolerant soybean worldwide (Barber et al., 2017). Some studies have demonstrated that dicamba applications in soybean at R2 (flowering) cause a higher yield loss compared with V3 soybean stage, considering the same concentration (Robinson et al., 2013; Solomon; Bradley, 2014; Anonymous, 2016a, 2016b). In another study, for 4.4 and 17.5 g ae ha^{-1} dicamba, soybean yield loss was around 2.5 times higher at R1 compared to V4 application (Griffin et al., 2013). Thus, due to the demand for studies on the effects caused by the dicamba off-target movement on non-tolerant soybean in brazilian conditions (cultivars and climate), these studies were conducted with the objective of determining the behavior and effect (injury and yield) of dicamba simulated drift in different soybean cultivars. The hypothesis of the work is the characteristics of different generations of soybean cultivars and the Brazilian climatic conditions can influence the damage caused by dicamba drift. The objective of this work was to evaluate the effect of drift at different levels in two developmental stages of different Brazilian cultivars.

MATERIAL AND METHODS

Three field experiments were carried out to estimate the effects of dicamba drift on non-tolerant soybeans.

Experiment I and II

Experiments I and II were carried out at Embrapa Soja Experimental Farm (23º11'37" S and 51º11'03" O), Londrina, Paraná, in 2017/2018 and 2018/2019 summer growing seasons, respectively, with the objective of testing concentrations that simulate a drift event in a soybean cultivar to identify the effects of low concentrations on the plant. Londrina has a warm humid subtropical climate, without dry season and hot summer, presenting an average temperature of 20.9°C and a yearly average precipitation of 1,429 mm (Figures I and II)

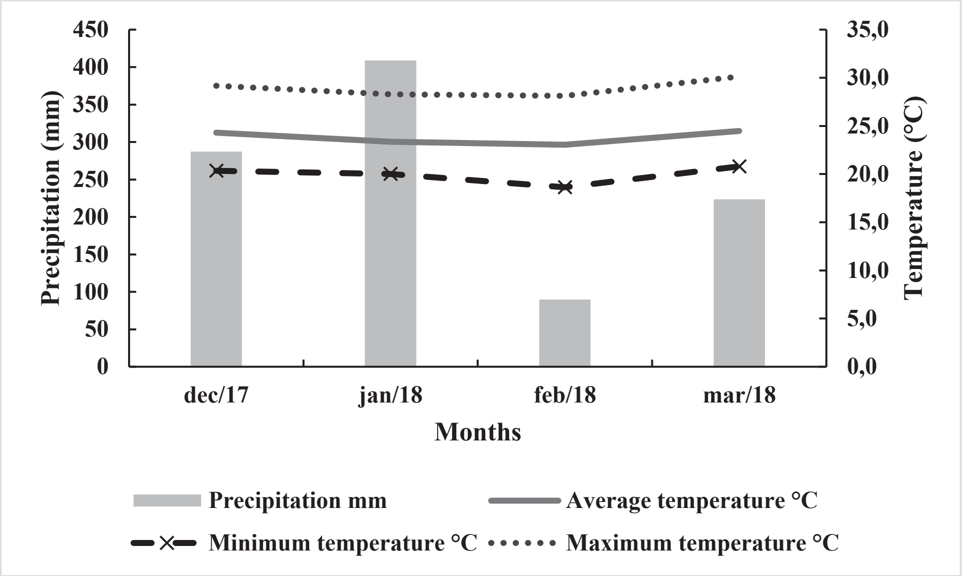

Figure 1 - Precipitation, maximum, minimum and average temperature for the Londrina region, where experiment I were carried out (National Institute of Meteorology, 2022).

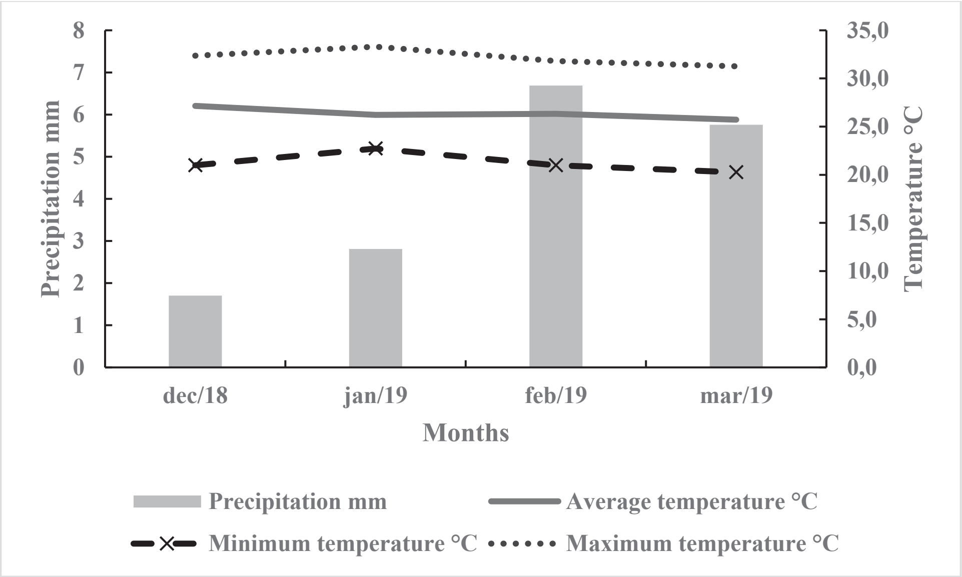

Figure 2 - Precipitation, maximum, minimum and average temperature for the Londrina region, where experiment II were carried out (National Institute of Meteorology, 2022).

Soybean was sown under no-tillage in all areas. The BRS 1010 IPRO cultivar, which is a dicamba non-tolerant soybean, was used at experiments I and II, sown in rows spaced 50 cm apart at 4x10 m plots, in a planting density of 14 plants per linear meter. Sowing was performed on November 6, 2017 for experiment I; on November 11, 2018 for experiment II.

The 0-20-20 (N-P-K) fertilizer was employed in all experiments, being applied at a fertilization rate of 200 kg ha^{-1} at experiment I, supplied 18 kg ha^{-1} of K_2O and 30 kg ha^{-1} of P_2O_5, and 150 kg ha^{-1} at experiment II, where 40 kg ha^{-1} of P_2O_5 and 24 kg ha^{-1} of K_2O were supplied.

The experiments were organized in a randomized complete block design with four replications. Experiment I treatments consisted of different simulated drift concentrations of dicamba herbicide (Atectra®, 480 g ae L^{-1}, BASF, SP, Brazil): 0, 1.87, 7.48, 30, 120 and 480 g ae ha^{-1}, which correspond, respectively, to 0%, 0.39%, 1.56%, 6.25%, 25% and 100% of Atectra® L ha^{-1} mean field concentration for weed control. These concentrations were used as a first experimental evaluation of dicamba drift effects in a soybean cultivar. Dicamba was applied in soybean crop at V3 (third node - second trifoliate leaf completely unfolded) and R1 (beginning of flowering – presence of flower at any node on main stem) stage of growth. As soybean sowing at Paraná, Brazil goes from September to January, drift to non-target area can be present at these both stages.

At experiment II, treatments consisted of dicamba application in V3 and R1 soybean growth stages, at ten application concentrations (Atectra®, 480 g ae L^{-1}, BASF, SP, Brazil): 0, 0.02, 0.04, 0.08, 0.15, 0.30, 0.6, 1.2, 2.4 and 4.8 g ae ha^{-1} which correspond, respectively, to 0%, 0.004%, 0.008%, 0.016%, 0.031%, 0.063%, 0.13%, 0.25%, 0.50% and 1% of Atectra® L ha^{-1} mean field concentration for weed control. These data were selected from the first experiment and observable drifts events resulted from experimental applications at different distances (data not show).

Herbicide applications were performed using a CO_2 pressurized backpack sprayer, with a 2-m boom width and four spray tips, under pressure of 241 kPa, calibrated to deliver 200 L ha^{-1} at 3.6 km h^{-1} application speed. For applications, AIXR 11002 nozzles (TeeJet, SP, Brasil) were used at experiments I and II. During application, conditions were: average temperature of 31.5°C, 85% relative humidity, and wind speed from 3 to 10 km h^{-1}, at experiment I; average temperature of 33°C, 37% relative humidity, and wind speed from 4 to 14 km h^{-1}, at experiment II.

Dicamba injury effects on soybean crop were assessed by estimating visible injury symptoms rated on the scale of 0 to 100%, where 0% means no foliar injury symptoms and 100% plant death (adapted from Behrens; Lueschen, 1979). Grades were assigned to the symptoms observed in the leaves of the plants on the selected dates, with the same two evaluators.

At experiment I, injury evaluation timings were reported for the V3 application, at 2, 9, 15 and 22 days after treatment (DAT); and for the R1 application, at 6, 13, 20 and 28 DAT. At experiment II, visible injury was evaluated at 7, 14, 21 and 28 DAT, for both V3 and R1 applications. At soybean physiological maturity, grain yield and plant height were assessed at experiment I and II.

Experiment III

Experiment III was conducted at Campos Gerais Agricultural Experiment Station (25º25'44,5" S e 50º03'13,0" O), Palmeira, Paraná, in 2018/2019 summer growing season, with the objective of testing the response of plants of different soybean cultivars after being exposed to a drift event. Palmeira presents humid subtropical climate without dry season and temperate summer, with an average temperature of 17.4°C and a yearly average precipitation of 1,476 mm (Aparecido et al., 2016). The climatic characteristics of region are shown in figure 2.

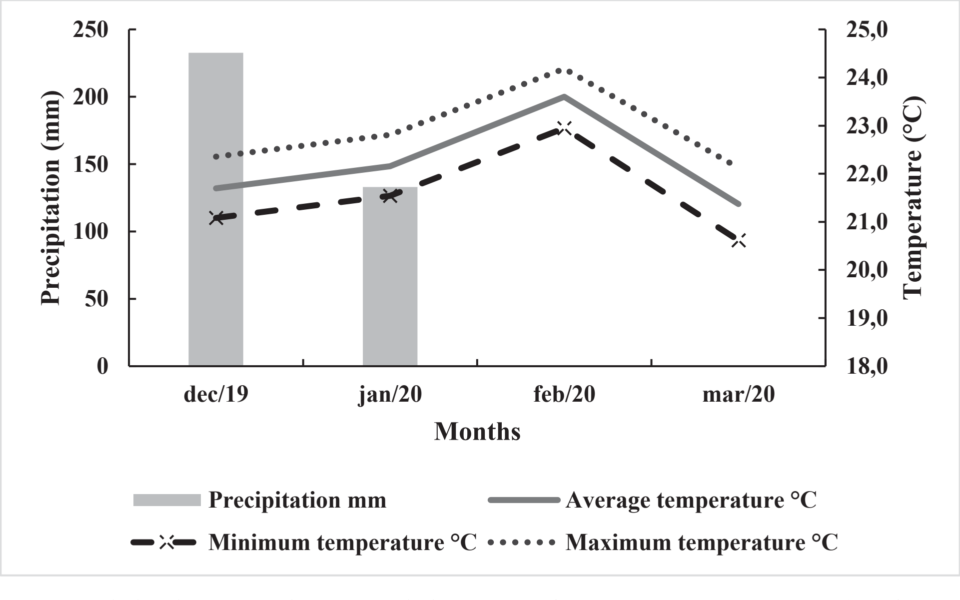

Figure 2 - Precipitation, maximum, minimum and average temperature for the Palmeira region, where experiment III was carried out (National Institute of Meteorology, 2022).

At Experiment III, rows were spaced 45 cm apart at 2x4 m plots, in a planting density of 16 plants per linear meter and the following cultivars were used: BRS 388 RR, BRS 399 RR, BRS 413 RR, BRS 433 RR, BRS 284, BRS 511, BRS 1001 IPRO, BRS

1003 IPRO and BRS 1007 IPRO, mixing glyphosate susceptible and tolerant cultivars, both from EMBRAPA cultivars collection (Table 1).

Table 1. Characteristics of the cultivars selected for Experiment III.

Cultivars	Technology	Maturity Group	Growth Habit	Cicle (days)	Other Features
BRS 388 RR	Glyphosate tolerant	6.4	Undetermined	116 a 128	High yield potential and excellent stability at different sowing times and high fertility environments;
BRS 399 RR	Glyphosate tolerant	6.0	Undetermined	101 a 110	Excellent health, combined with high productive potential even in areas with the presence of Nematodes (*Meloidogyne incognita* and *Meloidogyne javanica*).
BRS 433 RR	Glyphosate tolerant	6.0	Undetermined	117 a 124	Moderate resistance to root-knot nematode *Meloidogyne javani*
BRS 284	Conventional	6.3	Undetermined	119 a 121	Excellent productive potential also in areas with the presence of root-knot nematode *Meloidogyne javani*ca; Cycle and size that enable the 2nd corn crop.
BRS 511	Conventional	6.4	Undetermined	130 a 139	Broad indication adaptation, stability and moderate resistance to root-knot nematode *Meloidogyne javanica*; Great performance in early sowing (planting opening), in the various indication regions; Cultivar favorable to the management of Asian rust
BRS 1001 IPRO	Glyphosate tolerant; Technology INTACT	6.2	Undetermined	119 a 128	Better performance at the beginning of the sowing season; Excellent productive potential also in areas with the presence of root-knot nematode *Meloidogyne javanica* (early sowings)
BRS 1003 IPRO	Glyphosate tolerant; Technology INTACT	6.3	Undetermined	117 a 130	Excellent productive potential even in areas with the presence of root-knot nematode *Meloidogyne javanica*;
BRS 1007 IPRO	Glyphosate tolerant; Technology INTACT	6.0	Undetermined	117	Excellent productive potential even in areas with the presence of root-knot nematode *Meloidogyne javanica*;

Adapted by EMBRAPA SOJA, 2022.

Sowing was performed on December 12, 2018. The 0-20-20 (N-P-K) fertilizer was employed in all experiments, being applied at a fertilization rate of 200 kg ha^{-1} at experiment III, supplied 18 kg ha^{-1} of K_2O and 30 kg ha^{-1} of P_2O_5. In Paraná State, soybeans are cultivated from September to January; this planting window varies between regions of the state. Soil fertilization was carried out in the total area, with crop sowing, in the planting line.

At experiment III, treatments were arranged in a 9 x 3 factorial design, comprising nine soybean cultivars (before mentioned) and three application concentrations of dicamba (Atectra®, 480 g ae L^{-1}, BASF, SP, Brazil), selected based on information obtained in previous experiments: 0, 1.2 and 2.4 g ae ha^{-1}, which correspond to 0%, 0.25% and 0.50% Atectra® L ha^{-1} mean field concentration for weed control. Dicamba was applied only in soybean crop at V3 soybean stage of growth. The R1 stage was not applied.

Herbicide applications were performed using a CO_2 pressurized backpack sprayer, with a 2-m boom width and four spray tips, under pressure of 241 kPa, calibrated to deliver 200 L ha^{-1} at 3.6 km h^{-1} application speed, using AIXR11002-VP nozzles (TeeJet, SP, Brasil). During application, conditions were: average temperature of 22.5°C, 70% relative humidity, with wind speed from 2 to 6 km h^{-1}.

Experiment three also follows the same scale of injury assessment in plants (visual injury evaluation) and were taken at 15 and 30 DAT. Grain yield was assessed, also at physiological maturity stage, with grain moisture at 13%. Grain yield was estimated based on the plant population per hectare, the weight of 1,000 grains per treatment, the number of pods per plant and the number of grains per pod (Lee; Herbek, 2005; Castell, 2012). Plant height was measured from the insertion between stem and root to the length of the last trifoliate leaf fully developed.

Data from the three experiments were adjusted to sigmoidal and polynomial regression models and for better understanding, the injury assessments were divided into four weeks (one, two, three and four weeks after application). All data underwent a Shapiro-wilk normality test and the regression lines were adjusted with the aid of SigmaPlot (12.1). In experiment III data from different cultivars were submitted to analysis of variance (ANOVA) using AgroEstat software. When significant ($p \leq 0{,}05$), means were compared by Tukey test.

RESULTS AND DISCUSSION

In Experiment I, visible injury symptoms in soybean plants began to be observed in week one at V3 and R1 drift treatments (Figure 4). Simulated dicamba drift rates above 1.87 g ae ha^{-1} were enough to cause changes in leaf development. Soybean plants that were exposed to rates of 120 g ae ha^{-1} or higher at V3, died at week three. The same occurred for drift event at R1 for the 480 g ae ha^{-1} rate. From week four, non-death plants began to show recovery (rates below than 120 g ae ha^{-1}) regardless of plants growth stage respectively.

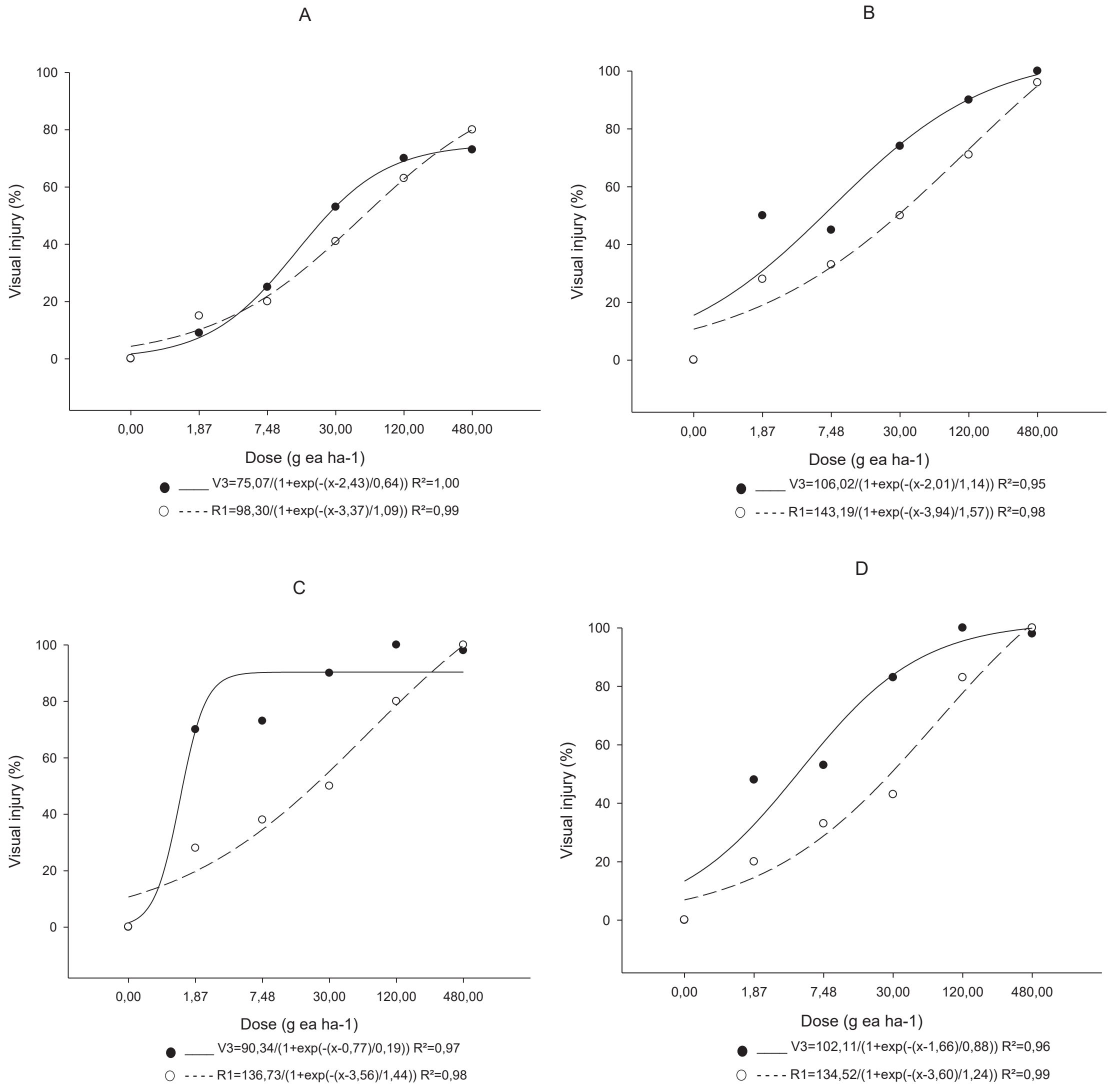

Figure 4 - Visual injury assessment (%) of the BRS1010 IPRO soybean in weeks after the application of different concentrations of dicamba in two phenological stages of development (V3 and R1). Embrapa Soja, Londrina / PR, 2018. A - week one; B - week two; C - week three; D - week four.

Dicamba drift caused greater visual injury effects on soybean in the vegetative stage (V3), compared to the reproductive stage (R1). Symptoms were observed in three to four fully developed trifoliate leaves after plants tissue were sprayed (Figure 4). Visual symptoms included cupping of trifoliate leaves, deformation of leaf edge, reductions in plant height and, in some cases, death of the apical bud. Injury in vegetative stages has been known to present a greater potential for soybean recovery due to, among other aspects, a longer time interval between the contact of the herbicide with the plant and the end of plant life cycle, minimizing interference on soybean yield (Jones et al., 2019a; Robinson et al., 2013).

In R1, dicamba drift caused higher visual injury effect starting from the 1.87 g ae ha^{-1} rate (Figure 4), demonstrating that this is a critical stage for potential exposure to dicamba. Several studies have documented that reproductive stages, especially flowering ones, are more vulnerable to yield loss compared to vegetative stages or late reproductive stages (Egan et al., 2014; Solomon, Bradley, 2014).

Soybean height and yield decreased as drift rates of dicamba increased, demonstrating an effect on these parameters (Figure 5), as observed by Auch, Arnold (1978), Silva et al. (2018), Jones et al. (2019c). Plant height and grain yield decreased up to 100%. Final height of the soybean was more negatively affected by dicamba application at R1, differently from what was observed for visual injury.

Reduction in plant height seems to be more common when herbicide exposure occurs in early reproductive stages, since, as plants get closer to maturity, they are near or already reached maximum height (Solomon, Bradley, 2014; Jones et al., 2019a, 2019b). However, in this experiment, the opposite was observed, possibly due to the indeterminate growth habit of soybean cultivars used in Brazil, which allows plants to continue to grow even after the beginning of the flowering. Robinson et al. (2013) explain that the reduction in height of plants treated with auxinic herbicides is caused by the accumulation of abscisic acid that can limit plant growth. According to Robinson et al. (2013) the reduction in height decreases leaf area and production of photoassimilates, resulting in lower yields.

As for grain yield, for both V3 and R1 drift events at 30, 120 and 480 g ae ha^{-1} rates, plants died, and thus, there was no grain production. Regarding the other drift rates, dicamba negatively affected grain yield if applied more than 30 g ae ha^{-1} in V3 and R1, i.e. 6.25 to 1.56% of the highest studied rate (1 L Atectra® ha^{-1}). Plants with up to 73% visible injury in week three in V3 drift events could recover and produce as much as plants that was not exposed to dicamba. However, in R1 drift event, soybean recovery was only possible for up to 28% visible injury. Although the visual injury effect of dicamba is very severe, yield will not always be reduced (Figure 5).

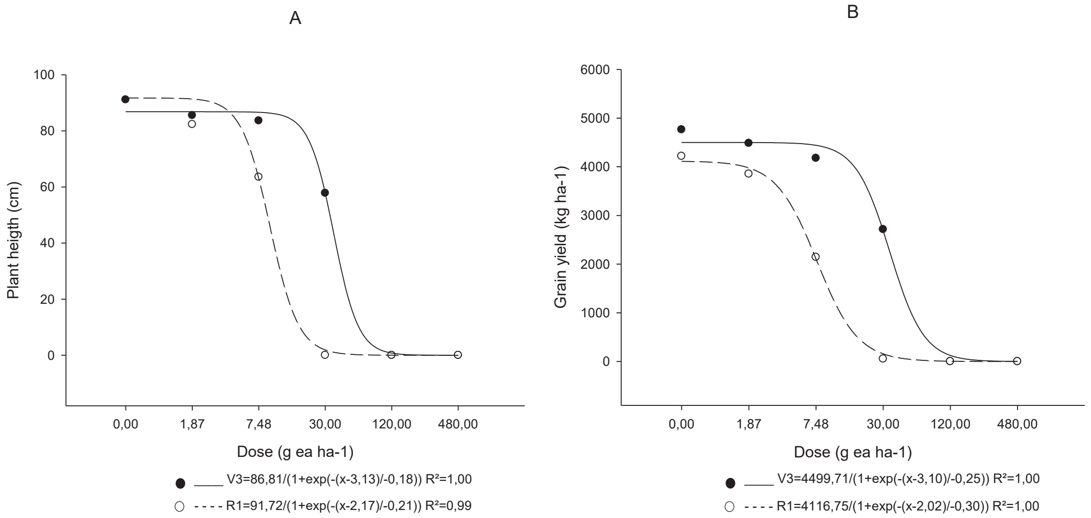

Figure 5 - Plant height (cm) and grain yield (kg ha^{-1}) of soybean BRS1010 IPRO in weeks after the application of different concentrations of dicamba in two phenological stages of development (V3 and R1). Embrapa Soja, Londrina/PR, 2018. A – plant heigth; B – grain yield.

In experiment II, lower rates of dicamba were used, for the same non-dicamba-tolerant soybean cultivar (BRS 1010 IPRO) as for experiment I. In V3 event, the visual injury symptoms in week one varied from 0 to 70%, tending to decrease in week four. In R1 event, the initial symptoms were again less severe and soybean plants showed recovery up to week four (Figure 6).

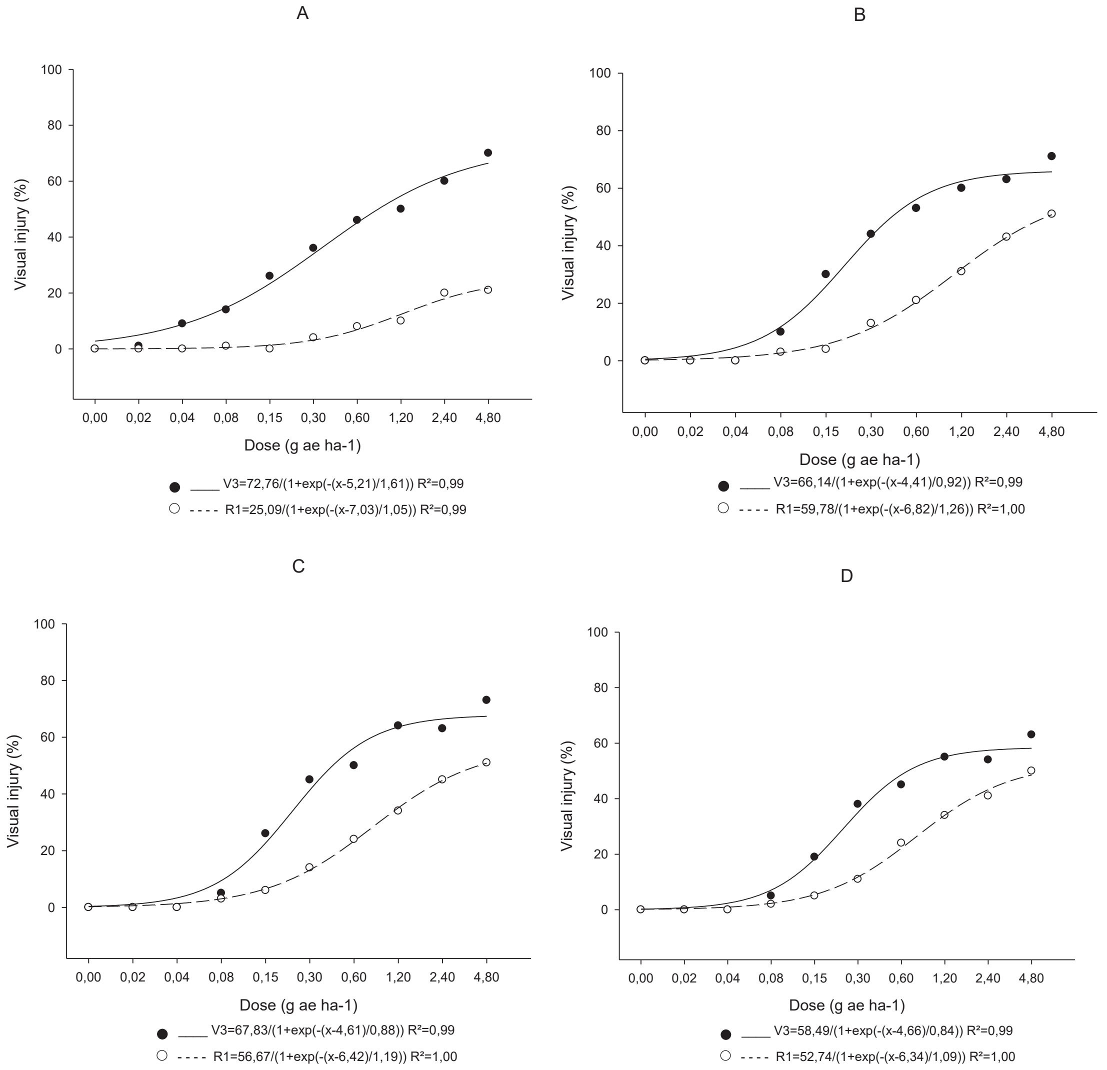

Figure 6 - Visual injury assessment (%) of the BRS1010 IPRO soybean in weeks after the application of different concentrations of dicamba in two phenological stages of development (V3 and R1). Embrapa Soja, Londrina / PR, 2018. A - week one; B - week two; C - week three; D - week four.

Soybean cultivars of indeterminate growth habit continue to present injury symptoms in newly formed leaves even being in reproductive stages (Heatherly, Elmore, 2004). In week four, it was observed that in both V3 and R1 events, dicamba rates of up to 0.08 g ae ha^{-1} did not differ in injury from the control treatment; and applications from 0.25% to 1% of the commercial concentration of the herbicide (4.8 g ae ha^{-1}), did not differ in V3 event. However, the 1% concentration was the one that most caused injury in R1 event (Figure 6).

Plant height decreased with the increase of application rates of dicamba in soybean at V3, which occurred starting from 1.2 g ae ha^{-1} (Figure 7). In R1 event, soybean height was only reduced starting from 2.4 g ae ha^{-1}, whereas in the Experiment I, from 1.87 g ae ha^{-1}. With concentrations close to one gram of the acid equivalent of dicamba, the effect of decreasing plant height may occur if the application occurs at early stages, demonstrating a high sensitivity of this characteristic to the active ingredient.

These results agree with those of Solomon, Bradley (2014) and Costa et al. (2020) who observed a reduction in plant height after exposure to dicamba at V3 and R2 stages. However, they demonstrate a correlation of these effects with environmental conditions in which plants are cultivated. As seen in Figure 7, height reduction and injury resulting from the dicamba drift in the vegetative stages do not always compromise crop yield, which was also reported by Al-Khatib, Peterson (1999). Grain yield was not affected when soybean was exposed to any dicamba rates at V3, i.e. plants contacted by dicamba rates of up to 4.8 g ae ha^{-1}, despite visual injury (up to 73%) and plant height reduction (up to 26.9%), did not reduce crop yield.

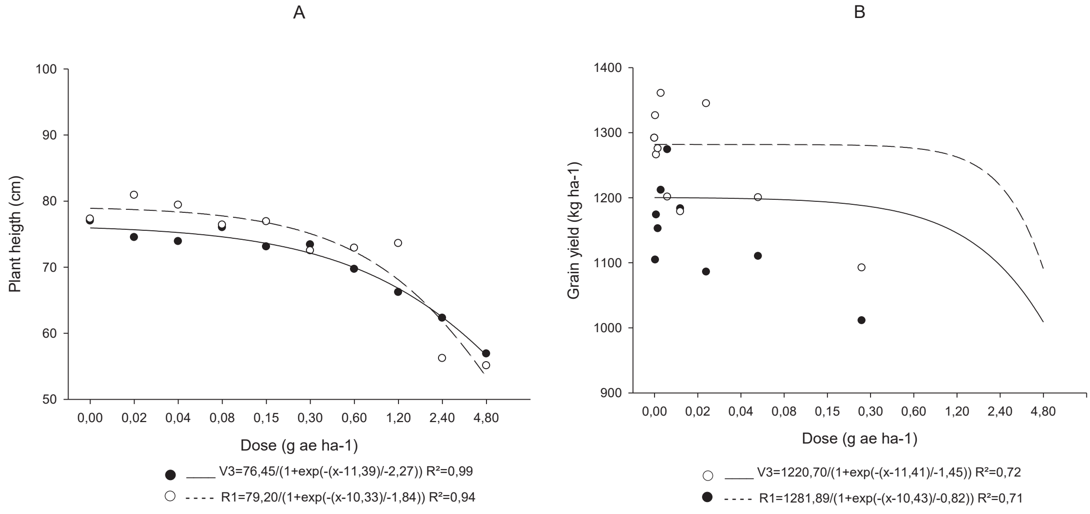

Figure 7 - Plant height (cm) and grain yield (kg ha^{-1}) of soybean BRS1010 IPRO in weeks after the application of different concentrations of dicamba in two phenological stages of development (V3 and R1). Embrapa Soja, Londrina/PR, 2018. A – plant heigth; B – grain yield.

In R1 event, only the highest dicamba rate 4.8 g ae ha^{-1} applied in soybean reduced grain yield compared to 0.02, 0.15 and 1.2 g ae ha^{-1} rates. No differences were observed among the other application rates for grain yield. A reduction in plant height, probably associated with a reduction in leaf area and a lower number of nodes in the main stem, would result in fewer pods and/or lighter grains weight due to reduction in photosynthesis. It should be noted that in the 2018/19 summer season there was a severe drought that may justify the low yields.

In week three (Table 2), the highest levels of injury were observed, varying from 38 to 68%. For both application rates, BRS 388 RR and BRS 399 RR cultivar showed higher sensitivity to dicamba, whereas BRS 511 cultivar showed lower sensitivity than BRS 388 RR and BRS 399 RR.

Table 2. Means from the visual injury assessment (%) in week three after treatment, for different application rates of dicamba and different soybean cultivars. Experiment III, Palmeira/PR, 2019.

Cultivars	Rate (g ae ha^{-1})	
	1.2	2.4
BRS 388 RR	61 Aa	68 Aa
BRS 399 RR	51 ABa	58 ABa
BRS 433 RR	46 BCa	49 BCa
BRS 413 RR	43 BCa	45 Ca
BRS 284	48 BCa	53 BCa
BRS 511	38 Ca	43 Ca
BRS 1001 IPRO	44 BCa	50 BCa
BRS 1003 IPRO	45 BCa	50 BCa
BRS 1007 IPRO	46 BCa	48 BCa
p	<0.001	

Means followed by the same letter within a column do not significantly differ by Tukey test a 5% level of significance. Uppercase letters compare soybean cultivar within herbicide rates (vertical disposition); lowercase letters compare herbicide rates within soybean cultivars (horizontal disposition).

In week four, in general, it was already possible to observe reduction in symptoms caused by dicamba injury (Table 3). For the 1.2 g ae ha^{-1} treatment, highest visible injury ratings were observed in BRS 388 RR, BRS 433 RR, BRS 284, BRS 511 and BRS 1003 IPRO cultivars. For the 2.4 g ae ha^{-1} treatment, BRS 388 RR differed from BRS 399 RR, BRS 413 RR, and BRS 511; whereas BRS 1001 IPRO showed less sensitivity to dicamba. On the two dates evaluated, no significant differences were observed for the visual assessment of injury between the two application rates of dicamba. JONES et al. (2019b) indicate that soybean is critically sensitive to dicamba when exposed to low rates in vegetative stages, either for DGA (Atectra®) or BAPMA (Engenia®) dicamba formulations.

Table 3. Means from the visual injury assessment (%) in week four after treatment, for different application rates of dicamba and different soybean cultivars. Experiment III, EECG, Palmeira/PR, 2019.

Cultivars	Rate (g ae ha^{-1})	
	1.2	2.4
BRS 388 RR	40 Aa	38 Aa
BRS 399 RR	25 BCa	23 Ba
BRS 433 RR	36 ABa	34 ABa
BRS 413 RR	25 BCa	23 Ba
BRS 284	29 ABCa	28 ABa
BRS 511	28 ABCa	23 Ba
BRS 1001 IPRO	21 Ca	24 Ba
BRS 1003 IPRO	28 ABCa	31 ABa
BRS 1007 IPRO	26 BCa	29 ABa
p	0.037	

Means followed by the same letter within a column do not significantly differ by Tukey test a 5% level of significance. Uppercase letters compare soybean cultivar within herbicide rates (vertical disposition); lowercase letters compare herbicide rates within soybean cultivars (horizontal disposition).

When the different soybean cultivars were analyzed individually, no differences in grain yield were observed for the application of dicamba and the control treatment. The average soybean grain yield in the field, without herbicide treatment, varied from 2,700 to 1,980 kg ha^{-1}. The applied rates of dicamba did not differ in terms of the effects on grain yields of all cultivars (Figure 8).

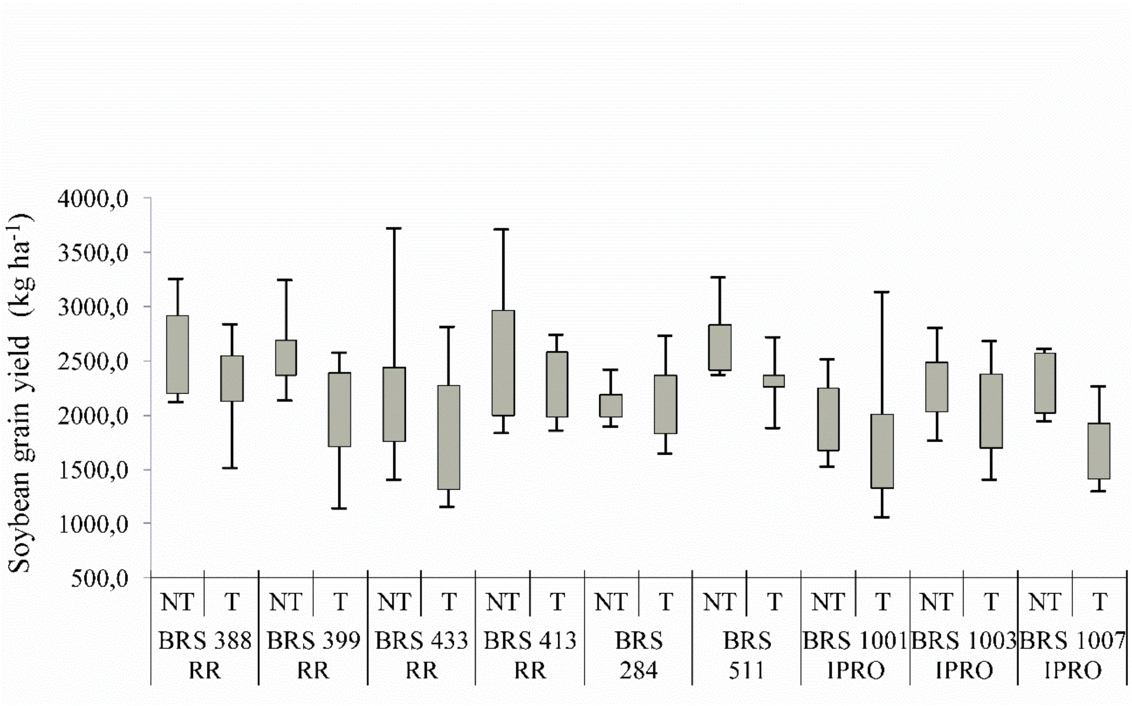

Figure 8 - Variability in soybean grain yield of non-treated (NT) and treated (T) soybeans with dicamba herbicide (average of 1.2 and 2.4 g ea of dicamba ha^{-1}, since they did not significantly differ by Tukey test a 5% level of significance), for the different cultivars.

As noted in this study, although dicamba causes severe injury symptoms in plants, yield losses can be minimal if only leaves and leaf petioles are affected. Higher yield losses are generally associated with reductions in the number of nodes, which limits the formation of reproductive structures (Weidenhamer et al., 1989). The herbicide rate and the stage of development of the plant at the time of the drift event will affect the degree of damage to yield (Costa et al., 2020).

As reported by Kelley et al. (2020), that a dicamba rate of 0.48 g ae ha^{-1} reduced grain yield by 6% when applied at V3 stage. For the cultivars, there was only a statistical difference between treatments with application of dicamba compared to the non-treated control, with an average reduction of 20% for up to 0.5% of the recommended concentration. There was no significant interaction between cultivars and dicamba rates, unlike what was observed by Robinson et al. (2013) that positively related soybean injury caused by dicamba with grain yield loss. Climatic conditions in Brazil and growth habit of the used soybean cultivars demand new experiments to address correlations between observed symptoms and yield losses caused by dicamba drift.

Visual injury effects of dicamba vary according to the growth stages of soybean and applied concentration. Soybean yield decreased as plants are exposed to increasing concentrations of dicamba. Injury is more visible in V3, although soybean yield was more affected by dicamba injury when applying it at R1. The sensitivity of soybean to dicamba was higher in cultivar BRS 388 RR at concentrations 1.2 and 2.4 g ae ha^{-1}, which may cause loss of productivity of up to 41%. The BRS 511 cultivar would be better suited to an eventual dicamba drift condition, as it showed less sensitivity to the application of the herbicide in concentrations 1.2 and 2.4 g ae ha^{-1}.

REFERENCES

Al-Khatib K, Peterson D. 1999. Soybean (*Glycine Max*) Response to Simulated Drift from Selected Sulfonylurea Herbicides. Weed Technol. 3:264–270.

Aparecido LEO, Rolim GDeS et al. 2016. Köppen, Thornthwaite and Camargo climate classifications for climatic zoning in the State of Paraná, Brazil. Ciência e Agrotecnol. 40(4):405-417.

Anonymous. Engenia® supplemental herbicide label. BASF Corporation, 2016a. [acessed 2020 Feb 18]. https://www.cdms.net/ldat/ldDG8007.pdf.

Anonymous. XtendiMax® supplemental herbicide label. Monsanto Corporation, 2016b. [acessed 2020 Feb 18]. https://www.cdms.net/ldat/ldDF9002.pdf.

Auch DE, Arnold WE. 1978. Dicamba use and injury on soybeans (Glycine max). Weed Sci. 26(5):471–475.

Alves GS et al. 2017. Spray Drift from Dicamba and Glyphosate Applications in a Wind Tunnel. Weed Technol. 31(3):387–395.

Bales SR, Sprague CL. 2020. Sensitivity of dry edible bean to dicamba and 2,4-D. Weed Technol. 34(1):117–124.

Barber LT et al. 2017. Dicamba in Arkansas - Frequently Asked Questions. University of Arkansas Research and Extension Publication FSA2181, 2017. [acessed 2020 Mar 19]. https://www.uaex.edu/publications/pdf/FSA-2181.pdf.

Behrens R, Lueschen W. 1979. Dicamba volatility. Weed Sci. 27(5):486–493.

Bradley KW. 2017. A final report on dicamba-injured soybean acres. [acessed 2020 Mar 19].https://ipm.missouri.edu/IPCM/2017/10/final_report_dicamba_injured_soybean/.

Bradley K. 2018. Dicamba injury update. Different year same questions. Integrated Pest and Crop Manage. [acessed 2020 Mar 19]. https://ipm.missouri.edu/IPCM/2018/7/July-15-Dicamba-injury-update-different-year-same-questions/.

Bunch TR et al. 2012. Dicamba Technical Fact Sheet. National Pesticide Information Center, Oregon State University Extension Services. [acessed 2019 Nov 4]. https://npic.orst.edu/factsheets/archive/ dicamba_tech.html.

Byker HP et al. Control of glyphosate-resistant horseweed (Conyza canadensis) with dicamba applied preplant and postemergence in dicamba-resistant soybean. Weed Technol. 27(3):492–496.

Cahoon CW et al. Palmer amaranth (Amaranthus palmeri) management in dicamba-resistant cotton. Weed Technol. 29(4):758–770.

Costa EM et al. 2020. Simulated drift of dicamba and 2,4-d on soybeans: Effects of application concentration and time. Biosci J. 36(3):857–864.

Egan JF, Barlow KM, Mortensen DA. 2014. A Meta-Analysis on the Effects of 2,4-D and Dicamba Drift on Soybean and Cotton. Weed Sci. 62(1):193–206.

Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA] 2022. Cultivares de soja – Centro-Sul do Brasil Macrorregiões 1, 2 e 3 e REC s 401 e 402. Embrapa soja - Londrina. 39p.

Foster MR et al. 2019. Development of a model to predict soybean yield loss from dicamba exposure. Weed Technol. 33(2):287–295.

Griffin JL et al. 2013. Soybean Response to Dicamba Applied at Vegetative and Reproductive Growth Stages. Weed Technol. 27(4):696–703.

Heap, I. 2020. The International Herbicide-Resistant Weed Database. [acessed 2020 Mar 15]. https://www.weedscience.org.

Heatherly LG, Elmore RW. 2004. Managing inputs for peak production: stem growth habit. Pages 454–458 in Boerma HR, Specht JE, eds. Soybeans: Improvement, Production, and Uses. 3rd ed. Madison, WI: American Society of Agronomy, Crop Science Society of America and Soil Science Society of America.

Jones GT et al. 2019a. Effect of low concentrations of dicamba alone and in combination with glyphosate on parent soybean and offspring. Weed Technol. 33(1):17–23.

Jones GT et al. Off-target movement of DGA and BAPMA dicamba to sensitive soybean. Weed Technol. 33(1):51–65.

Jones GT, Norsworthy JK, Barber T. 2019. Off-target movement of diglycolamine dicamba to non-dicamba soybean using practices to minimize primary drift. Weed Technol. 33(1):24–40.

Kelley K, Riechers D, Nordby D, Hager A. 2020. Plant growth regulator injury to soybean. [acessed 2020 Mar 14]. https://weeds.cropsci.illinois.edu/extension/factsheets/PGR.pdf.

Kniss AR. 2018. Soybean Response to Dicamba: A Meta-Analysis. Weed Technol. 32(5):507–512.

Kruger GR, Davis VM, Weller SC, Johnson WG. 2010. Control of horseweed (Conyza canadensis) with growth regulator herbicides. Weed Sci. 24(4):425–429.

Mccauley CL, Mcadam SAM, Bhide K, Thimmapuram J, Banks JA, Young BG. 2020. Transcriptomics in Erigeron canadensis reveals rapid photosynthetic and hormonal responses to auxin herbicide application. J Exp Bot. 71(12):3701-3709.

Maybank J, Yoshida K, Grover R. 1978. Spray drift from agricultural pesticide applications. Air Pollut Control Assoc J. 28:1009-1014.

Mueller TC, Steckel LE. 2019. Dicamba volatility in humidomes as affected by temperature and herbicide treatment. Weed Technol. 33(4):541–546.

Norsworthy JK et al. Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci. 60:31-62.

Peixoto AB. 1991. Tratos culturais químicos. STAB. 9(3):18-21.

Robinson AP, Simpson DM, Johnson WG. 2013. Response of Glyphosate-Tolerant Soybean Yield Components to Dicamba Exposure. Weed Sci. 61(4):526–536.

Robinson AP et al. 2013. Response of soybean yield components to 2,4-D. Weed Sci. 61(1)68–76.

Silva DRODa et al. 2018. Drift of 2,4-D and dicamba applied to soybean at vegetative and reproductive growth stage. Ciênc Rural. 48(8):1–7.

Solomon CB, Bradley KW. 2014. Influence of Application Timings and Sublethal Concentrations of Synthetic Auxin Herbicides on Soybean. Weed Technol. 28(3):454–464.

Wax LM, Knuth LA, Slife FW. 2020. Response of soybeans to 2,4-D, dicamba and picloram. Weed Sci. 17(3):388–393.

Weidenhamer JD, Triplett GBJr, Sobotka FE. 1989. Dicamba injury to soybean. Agron J. 81(4):637–643.

Wolf TM, Grover R, Wallace K, Shewchuk SR, Maybank J. 1992. Effect of protective shields on drift and deposition characteristics of field sprayers. In The Role of Application Factors in the Effectiveness and Drift of Herbicides. Agric. Canada. 29-52.

Chapter II

SOYBEAN INJURY FROM DICAMBA VOLATILITY AS INFLUENCED BY SPRAY ADDITIVES

ABSTRACT

Dicamba is often applied by farmers in combination with glyphosate in tank mixtures to increase efficacy or broaden the spectrum of weed control. This study evaluated soybean injury from dicamba volatility as a result of mixing different glyphosate formulations and a volatility reducing agent with dicamba. Three experiments were conducted in the 2019/2020 season in a randomized block design with four replications. Experiment I comprised ten treatments, including dicamba DGA salt mixtures with different glyphosate formulations (ammonium, dimethylamine, potassium, di-ammonium) with/without a volatility reducing agent, plus control treatment. In Experiment II, six treatments included mixtures of dicamba DGA salt with ammonium and potassium formulation of glyphosate salt with/without volatility reducing agent. Experiment III included BAPMA dicamba salt mixed with four glyphosate formulations (ammonium, dimethylamine, potassium, di-ammonium) plus control treatment. Herbicides were applied in boxes outside the plot and placed inside low tunnels between soybean rows for 48 h, and then removed. Soybean injury was evaluated 25 days after application and plant height, number of branches, and grain weight per plant were measured at crop harvest. Highest injury was observed for the mixture of dicamba and glyphosate ammonium salt regardless of the addition of a volatility reducing agent in Experiment I. In Experiment III, injury was higher with the mixture of BAPMA dicamba salt and glyphosate ammonium salt. Thus, to reduce volatility and injury to soybeans, dicamba should not be mixed with ammonium salt of glyphosate and a volatility reducing agent should be used. The mixture of dicamba and glyphosate potassium salt had the minor impact on herbicide volatilization.

Keywords: diglycolamine, N,N-Bis (3-aminopropyl) methylamine, ammonium salt, volatility reducing agent, off-target movement

INTRODUCTION

The recurrent use of the same herbicide can cause changes in the weed community composition selecting resistant weed biotypes (Norsworthy et al., 2012; Mueller, Steckel, 2019). One recent solution for the management of herbicide-resistant weeds in soybean and cotton production systems in North America and Brazil has been the deployment of genetically modified crop varieties tolerant to auxinic herbicides (e.g., dicamba, 2,4-D) (Bish, Bradley, 2017; Werle et al., 2018; Jones et al., 2019).

Considering the introduction of genetically modified soybean cultivars tolerant to auxinic herbicides, tolerance to dicamba will allow its use with greater flexibility and reduce the residual period between soybean burdown and sowing or facilitate its application in post-emergence of weeds within the crop (Kruger et al., 2010; et al., 2015; Sousa Alves et al., 2017).

Auxinic herbicides can cause abnormalities in broadleaf plants even at low concentrations, such as leaves wrinkling and cupping, chlorosis, epinasty, low plant development, and other symptoms (Costa et al., 2020). These symptoms can result from the use of incorrect application technology, which can move the herbicides off-target, either by drift at the time of application or even after application, by a secondary movement called volatilization (Mueller et al., 2013; Egan, Mortensen, 2012; Jones, 2018; Costa et al., 2020).

Cases of off-target injury caused by dicamba have been widely reported in recent years and are generally attributed to non-recommended spray nozzles, which produce small droplets that are suspended in the air after application, particularly under heat inversion conditions (Anonymous, 2018a, 2018b) or spray tank cleaning failures (Growe, 2017). However, these reports can also be related to product volatilization (Bish, Bradley, 2017; Hager, 2017). Dicamba is a weak acid and its molecular state (protonated or deprotonated) changes with the pH of the medium, which may substantially influence its volatility (MacIness, 2017).

Several authors have reported the volatility of different dicamba salts and that the product formulation alters this possibility (Behrens, Lueschen, 1979; Egan, Mortensen, 2012; Bish, Bradley, 2017). Two dicamba formulations are currently in the market, including dicamba diglycolamine (DGA) and N,N-Bis (3-aminopropyl) methylamine (BAPMA) dicamba salt. Some of the commercial formulations rely on the addition of a pH modifier, volatility reducing agent (VRA), acetate acid, commercially known as VaporGrip, to reduce product volatility (Hemminghaus et al., 2017). VaporGrip technology reduces the potential of volatility of dicamba salt, as it increases the pH of the solution (Hemminghaus et al., 2017).

In addition to the salt, the mixture of products also modifies the pH of the solution, including the mixture with glyphosate, used to increase the spectrum of weed control (Jones et al., 2018). According to Striegel et al. (2020) previous studies document the effect of glyphosate on the pH and volatility of different dicamba formulations. In another study by Mueller and Steckel (2019), the mixture of dicamba DGA salt and glyphosate was considered to be of high risk for dicamba volatility because the addition of glyphosate decreased the pH of the solution.

In Brazil, the Intacta XTend platform (technology approved in Brazil that allows the use of dicamba in soybean burdown application in cultures with Xtend technology) recommends potassic salt glyphosate as formulation for use in a tank mixed with dicamba (Monsanto Brazil). In the USA, glyphosate dimethylamine, isopropylamine and ammonia salts are not recommended to be included in a tank mix with dicamba for soybeans (Anonymous, 2020). With the greater adoption of the no-till system in soybean, corn and cotton, part of the dicamba will be deposited on straw and this is a problem because the herbicide dynamics with a straw cover surface is different from the soil surface or the surface foliar (Carbonari et al., 2020).

Therefore, based on this evidence, research is warranted to assess any negative effects of dicamba volatilization. The hypothesis of this work is that the mixture between dicamba and different glyphosate salts increases the damage caused by spray solution volatilization in sensitive soybeans. The present study was conducted with the objective of evaluating the influence of the mixture of different formulations and glyphosate salts with or without the addition of a VRA on the volatilization of different dicamba salts in non-herbicide tolerant soybean, and investigating the effect of these volatilized products on the crop development and productivity.

MATERIAL AND METHODS

Setting and plant material

Three field experiments were conducted during the 2019/2020 field season to determine the effects of dicamba volatilization on soybean. Experiments I and III were established at the Campos Gerais Agricultural Experimental Station (25º25'44.5"S and 50º03'13.0"W), Palmeira-Paraná, Brazil, and Experiment II at Embrapa Soja (23º11'37"S and 51º11'03”O), Londrina-Paraná, Brazil. Figures 1 and 2 demonstrate the climatic conditions in the two regions during the conduction of the experiments.

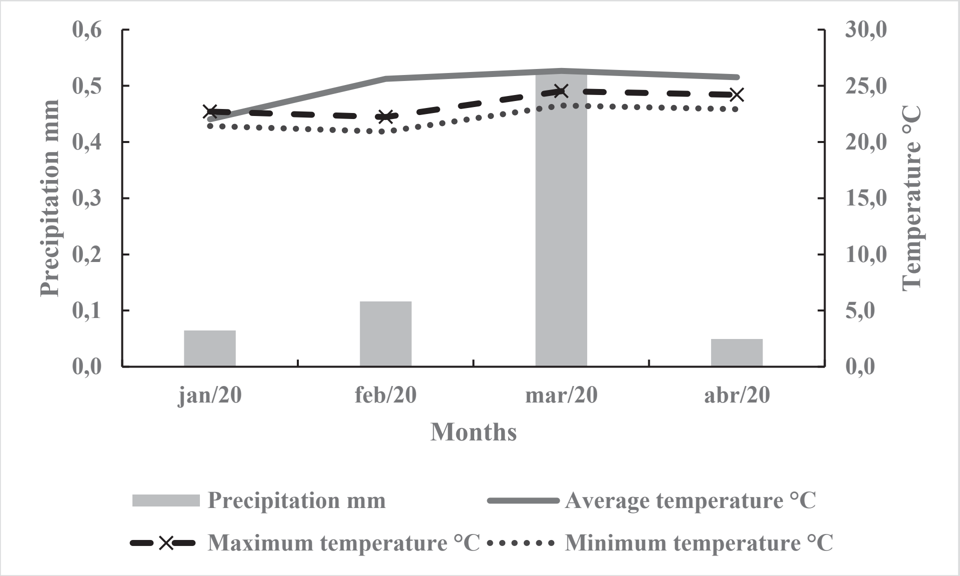

Figure 1 - Precipitation, maximum, minimum and average temperature for the Palmeira region, where experiment I and III were carried out (National Institute of Meteorology, 2022).

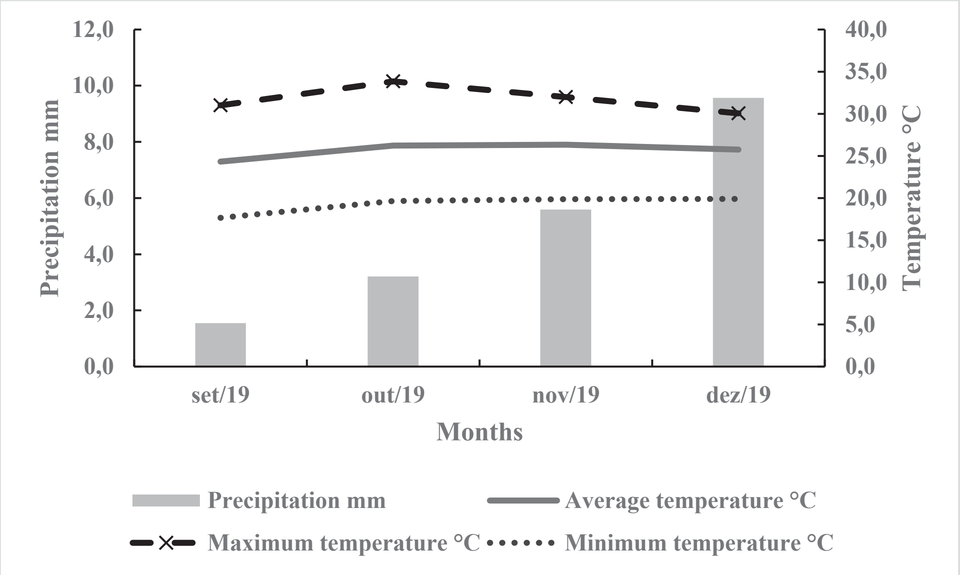

Figure 2 - Precipitation, maximum, minimum and average temperature for the Londrina region, where experiment II were carried out (National Institute of Meteorology, 2022).

In Experiments I and III, the soybean cultivar TMG 7063 IPRO, not tolerant to dicamba, was no-till planted in 3×5 m plots with 0.50 m row spacing, 5 cm depth, and 266.666 seeds per hectare. This 6.3 maturation group cultivar has an indeterminate growth habit and seeds were treated with Methyl N-{2-[1-(4-chlorophenyl)-1H-pyrazol-3 - yloxymethyl]phenyl}(N-methoxy) Carbamate + Dimethyl 4,4’-(o-phenylene)bis(3-

thioallophanate) + (RS)-5-amino-1-(2,6-dichloro-α-α-α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile (Standak Top®, BASF©, SP, Brazil) at a concentration of 0.2 L 100 kg^{-1} seeds and with Methyl benzimidazol-2-ylcarbamate + Tetramethylthiuram disulfide (Novozymes Bioag©, PR, Brazil) at 0.2 L 100 kg^{-1} seeds before planting. The experimental area received base fertilization with 320 kg ha^{-1} of 04-16-08 formulation (NPK). In Experiment II, soybean cultivar BRS 543 RR from maturity group 6.0, indeterminate growth habit, and not tolerant to dicamba was sown in a no-tillage system with 0.45 m row spacing; it was previously treated with Methyl benzimidazol-2-ylcarbamate + Tetramethylthiuram disulfide (Bayer©, SP, Brazil), a contact and systemic fungicide of the Benzimidazole and Dimethyldithiocarbamate groups, at 200 mL 100 kg^{-1} seeds.

Treatments and application

All experiments were conducted in a randomized complete block design with four replications. Treatments are described in Table 1. Four times the label rates used for these experiments. That is standard procedure in this type of experiments (Striegel et al., 2021). Herbicide concentrations used were the label concentrations multiplied by four to ensure and maximize the effects of dicamba volatilization. Injuries caused by auxinic herbicides over small concentrations are quite characteristic; however, at high concentrations, necrosis and death are very general definitions (Strachan et al., 2013; Mueller, 2015).

Table 1. Description of treatments in Experiments I, II, and III and their concentrations.

Treatment	Dicamba	Glyphosate salt	Volatility reducing agent	Concentrations			pH spray solution
				Dicamba (CP ha^{-1})	Glyphosate (CP ha^{-1})	Volatility reducing agent (%)	
Experiment I	nontreated control						6.00
	DGA[1]			4			6.40
	DGA		+	4		0.50	6.39
	DGA	Ammonium		4	5.32		3.33
	DGA	Ammonium	+	4	5.32	0.50	5.27
	DGA	Dimethylamine		4	8		5.71
	DGA	Dimethylamine	+	4	8	0.50	5.43
	DGA	Potassium		4	8		4.49
	DGA	Potassium	+	4	8	0.50	5.30
	DGA	Di-ammonium		4	10.4		5.46
	DGA	Di-ammonium	+	4	10.4	0.50	5.40
Experiment II	non treated						6.00
	DGA			4			6.40
	DGA		+	4		0.50	6.39
	DGA	Potassium		4	8		4.49
	DGA	Potassium	+	4	8	0.50	5.30
	DGA	Ammonium		4	5.32		3.33
	DGA	Ammonium	+	4	5.32	0.50	5.27
Experiment III	non treated						6.00
	BAPMA[2]			3.2			6.30
	BAPMA	Ammonium		3.2	5.32	-	6.24
	BAPMA	Dimethylamine		3.2	8	-	4.65
	BAPMA	Potassium		3.2	8	-	6.41
	BAPMA	Ammonium		3.2	10.4	-	4.54

[1] diglycolamine dicamba salt; [2] N,N-Bis (3-aminopropyl) methylamine dicamba salt.

Herbicide application was performed in 0.40×0.30×0.13 m (L×W×H) polystyrene boxes in Experiments I and III, and in 0.50×0.35×0.16m (L×W×H) cardboard boxes internally coated with plastic in Experiment II. Both box types were filled with soil from the experimental area and irrigated before application to field capacity. A CO_2 pressurized backpack sprayer was used with a 2 m boom and four spray nozzle type AIXR110015 (TeeJet, Cotia, São Paulo, Brazil) in Experiment II. In Experiments I and III, nozzle TTI11002 (TeeJet, Cotia, São Paulo, Brazil) with an application speed of 3.6 km h^{-1} and spray volume of 200 $L{\cdot}ha^{-1}$ was used. The herbicides were applied 1 km from the experiment, avoiding any herbicide drift to the area. Soon after spraying, the boxes were taken into low tunnels in the field. The pH of spray solutions was measured for the applying products before spray at experiment I and III.

The low tunnels (Figure 1) were built of a transparent material using plastic greenhouse film and fiberglass arches for the herbicides to volatilize at their maximum capacity. The low tunnels measured 2.0×1.0×0.7 m (L×W×H) in the first experiment and 3.0×1.0×0.7 m (L×W×H) in the second. The low tunnels were positioned in the center of the plot and covered two lines of soybean cultivation in a three trifoliate soybean. The low tunnels were installed before placing the boxes on one of their sides. After application, the boxes (repetitions) were brought to the experimental area and placed between the two rows of soybeans ensuring no contact of the soybean plants with the treated boxes. Soon after, ~5 minutes, the low tunnels were completely closed enhancing a microenvironment for herbicide volatilization. In Experiment II, the low tunnels were open and formed a wind corridor in the planting lines. Forty-eight hours after closing, the low tunnels were opened and the structures, including the boxes, were removed.

Figure 1 - Representation of the low tunnel and the arrangement of the soil boxes inside it.

Data collection and statistical analysis

In both experiments, soybean injury was evaluated at 25 days after application (DAA). Visual scores were attributed to symptoms in the two central soybean rows, that where closest to the boxes, ranging from 0% to 100% where 0% represented the absence of symptoms and 100% indicated the death of plants (adapted from Behrens and Lueschen, 1975). At the time of crop physiological maturity, the height of the soybean, number of branches, and weight of grains per plant were measured from eight plants per plot. These plants were randomly collected from the two rows under the low tunnels; avoiding plants from the direct area surrounding the boxes. The data obtained were subjected to analysis of variance; if treatments were significant ($p \leq 0.05$), they were subjected to the Scott-Knott test analysis at 5% probability for comparison of means. Each experiment was analyzed separately as they presented different results from observed data.

RESULTS

In Experiment I using dicamba DGA salt, the addition of the VRA to the solution reduced the injury effects observed in soybeans without glyphosate treatment after 25 days. This observation was different from that reported for any mixtures between the herbicides (Table 2). The highest injury values were found for the mixture of dicamba and ammonium salt glyphosate, and the lowest injury was detected for the mixture of dicamba and potassium salt glyphosate.

Table 2. Mean injury (%) mean plant height, number of branches, and grain weight per plant observed in soybean in 25 days after application (DAA), mean plant height, number of branches, and grain weight per plant of different mixtures containing dicamba diglycolamine salt (DGA), volatility reducing agent (+ or -), and glyphosate salts. Experiment I, Palmeira-PR, Brazil, 2020.

Volatility Reducing agent	Dicamba salt + glyphosate salt	Injury of the crop (%)*	Height (cm)	No. branches (unit)	Grain/plant weight (g)
	nontreated	-	65.75 A	2.84 B	11.59 A
-	DGA	10.00 B	57.39 B	4.30 A	11.48 A
+	DGA	8.75 C	58.27 B	4.09 A	12.20 A
-	DGA + Ammonium	13.75 B	52.72 C	4.84 A	10.67 A
+	DGA + Ammonium	18.75 A	55.09 C	4.46 A	10.18 A
-	DGA + Dimethylamine	16.67 A	53.94 C	3.87 A	10.43 A
+	DGA + Dimethylamine	10.00 B	58.05 B	4.06 A	10.65 A
-	DGA Di-ammonium	20.00 A	58.64 B	3.94 A	11.60 A

+	DGA Di-ammonium	21.25 A	51.76 C	4.87 A	10.13 A
-	DGA + Potassium	8.75 C	59.86 B	3.69 A	11.85 A
+	DGA + Potassium	5.00 C	63.68 A	2.69 B	11.55 A
p-value		0.0023	0.0034	0.0053	0,9063

+ volatility reducing agent addition; Means followed by the same lowercase letter in the column do not differ by the Tukey's mean test at 5%. *Injury was accessed in relation with a low tunnel soybean without herbicide.

The injury observed in soybean was lower in the presence of the VRA in the mixture of dicamba and glyphosate dimethylamine salt. The reducer agent had no effect in the groups treated with dicamba isolated or other glyphosate salts mixtures. The lowest injury values resulted from the application of dicamba with glyphosate potassium salt with or without VRA, dicamba with dimethylamine salt and VRA, and dicamba only.

Were evaluated the characteristics of soybean at harvest, and found that only the mixture of dicamba, VRA, and glyphosate potassium salt did not affect the plant growth as compared to the control treatment (Table 2). The number of branches was higher in the plants exposed to treatment with all mixtures of dicamba and glyphosate, except for the mixture of dicamba DGA plus glyphosate potassium salt, demonstrating stimulation of plant branching. Grain weight per plant did not differ between treatment groups.

In Experiment II with DGA formulation of dicamba, the addition of the VRA decreased the observed injury of dicamba alone as well as that of the mixture of dicamba and glyphosate potassium salt in all periods evaluated (Table 3). The highest injury was observed in soybean from the volatilization of the mixture of dicamba DGA and ammonium salt glyphosate, and the lowest values were observed for the soybean treated with dicamba alone or with the mixture of dicamba and glyphosate potassium salt along with the VRA.

Table 3. Mean injury (%), mean plant height, number of branches, and grain weight per plant observed in soybean in 25 days after application (DAA), mean plant height, number of branches, and grain weight per plant of different mixtures containing dicamba diglycolamine salt (DGA), volatility reducing agent (+ or -), and glyphosate salts. Experiment II, Londrina-PR, Brazil, 2020.

Volatility Reducing agent	Dicamba salt + glyphosate salt	Injury of the crop (%)	Height (cm)	No. Branches (unit)	Grain/plant weight (g)
	nontreated		105.80 A	3.10 A	25.30 A
-	DGA	18.80 C	105.30 A	3.20 A	24.90 A
+	DGA	6.30 D	102.70 A	3.10 A	27.50 A
-	DGA + Ammonium	45.00 A	56.00 C	3.80 A	21.00 A
+	DGA + Ammonium	46.30 A	84.00 B	3.80 A	24.90 A
-	DGA + Potassium	31.30 B	100.40 AB	3.50 A	24.20 A
+	DGA + Potassium	5.00 D	102.50 AB	3.70 A	22.50 A
p-value		<0.0001	<0.0001	1.8919	6.3355

+ volatility reducing agent addition; Means followed by the same lowercase letter in the column do not differ by the Tukey's mean test at 5%. *Injury was accessed in relation with a low tunnel soybean without herbicide.

The characterization of the symptoms observed revealed that the lowest injury values were attributed to the slight reduction in the general growth of the plants and the cupping of the youngest trefoils. The plots with higher injury values, in addition to the symptoms described above showed increased lateral budding in the stem buds; apical bud death was reported in some plants.

Differences between treatments were also be observed in the soybean architecture and production at the time of grain maturation. Plant height reduced when exposed to the volatilized agents from the mixture of dicamba and ammonium salt glyphosate with or without the VRA (Table 3). No change was observed in the number of branches and the weight of grains per plant in this case.

In Experiment III using BAPMA dicamba salt, the mixture of dicamba with ammonium or di-ammonium salt of glyphosate induced higher injuries when compared to other glyphosate formulations.

Table 4. Mean injury (%), mean plant height, number of branches, and grain weight per plant observed in soybean in 25 days after application (DAA), mean plant height, number of branches, and grain weight per plant of different mixtures containing dicamba N,N-Bis (3-aminopropyl) methylamine dicamba salt (BAPMA), volatility reducing agent (+ or -), and glyphosate salts. Experiment III, Palmeira-PR, Brazil, 2020.

Dicamba salt + glyphosate salt	Injury of the crop (%)	Height (cm)	No. branches (unit)	Grain/plant weight (g)
		65.75 A	2.84 B	9.18 A
BAPMA	18.75 B	54.64 B	3.37 B	8.98 A
BAPMA + Ammonium	27.50 A	48.86 C	4.53 A	10.29 A
BAPMA +Dimethylamine	20.00 B	57.31 B	4.22 A	11.39 A
BAPMA +Di-ammonium	25.00 A	49.92 C	5.06 A	11.59 A
BAPMA + Potassium	18.75 B	62.43 A	3.65 B	9.82 A
p-value	0.0657	0.0006	0.0055	0.1741

+ volatility reducing agent addition; Means followed by the same lowercase letter in the column do not differ by the Tukey's mean test at 5%. *Injury was accessed in relation with a low tunnel soybean without herbicide.

We analyzed the components of soybean yield and found that the plant height decreased when dicamba was used, except with the mixture of dicamba and glyphosate potassium salt (Table 4). The decrease in the plant height was more pronounced in the presence of ammonium and di-ammonium salt glyphosate. The number of branches was higher in the group exposed to the mixtures with ammonium salt, dimethylamine, and di-ammonium salt than in the control and dicamba alone treatment groups; the weight of grains per plant did not differ between treatments.

Analyzing the pH of spray solutions, all the dicamba solutions had very similar pH values, indicating that pH may have no role in the product volatilization observed data. Among the mixtures with glyphosate, the ammonium salt of glyphosate formulation caused a greater decrease in the pH of the solutions, as the potassium salt, contrasting with differences observed from crop injury. In relation to BAPMA dicamba salt, the mixture with glyphosate dimethylamine induced a maximum decrease in the pH by 26% among the solutions analyzed.

DISCUSSION

The highest injuries in soybean resulting from the volatilization of the applied agents were mediated by the mixture of dicamba and ammonium salt glyphosate with or without the use of the VRA in Experiments I and II, indicating that this association was most likely to generate problems for soybean.

Acoording Jones et al. (2019) severe injuries resulting from primary and secondary drift at dicamba DGA application sites than from BAPMA dicamba application. Soltani et al. (2020) observed injuries in soybean sensitive to dicamba upon primary and secondary movement of the herbicide. According to these authors, injuries were detected in soybean kept covered by canvas for a period of 1 hour after the application of the herbicide. This injury was up to 50% and attributed to herbicide volatilization. Jones et al. (2018) reported soybean leaf malformation 14 days after the application of dicamba mixed with glyphosate. At 28 days after treatment, dicamba alone or with glyphosate did not cause any difference in the injuries observed in the crop.

Experiment II corroborates the results found in Experiment I, also signaling that the non-addition of the VRA can cause greater damage to the environment from dicamba DGA salt, even in a solution with potentially less problematic glyphosate salt, as is the case with potassium salt that contributed to 62% increase in crop injury after exposure to the spray solution. The use of the VRA decreased the injury to the crop by up to 37% in Experiment I when mixed only with dicamba DGA salt. In Experiment II, this reduction was up to 80%. The worst situation was observed when dicamba was mixed with ammonium salt of glyphosate, consistent with higher injury levels in the crop. The VRA is essential to reduce the risk of injury in crops caused by the volatilization of dicamba.

In Experiment III, although the VRA was not applied, an alternative dicamba salt with a lower potential for volatilization was used. The results showed higher injury of the BAPMA dicamba salt in the presence of the ammonium salt of glyphosate, indicating the higher possible risk of its volatility when mixed with ammonium salts.

Maximum crop injury up to 21 DAA, with significant height loss in the presence of higher concentrations of dicamba were observed in others studies (Jones et al., 2019). However, Bauerle (2014) highlights the ability of soybeans to recover from significant injuries caused by dicamba without suffering yield loss, which is important for the adoption of dicamba-tolerant technology.

A higher concentration of dicamba in the air when mixed with ammonium sulfate, used as an adjuvant, and glyphosate dimethylamine salt, two components that can increase the potential for volatility of dicamba (Sall et al., 2020). According to these authors, the concentration of dicamba in the air exponentially dropped after 72 hours of application under the study conditions. This observation demonstrates its little persistence in the air, with the maximum flow occurring in the first 24 hours after application. Total volatile losses among all formulations and study conditions ranged from 0.023% ± 0.003% to 0.302% ± 0.045% of the applied dicamba.

Studies show that even adding potassium salt glyphosate to dicamba, volatilization does not increase for some surfaces, such as glass and wet soil. In the same study, for all treatments and surfaces, the addition of a volatility reducing agent significantly reduces volatilization, which demonstrates the need for its use in tank mixing (Carbonari et al., 2020). The drift of dicamba vapor after spraying and its deposition can be controlled by formulating dicamba (Sharkey et al., 2020), or by adding additives to the tank mix to reduce volatilization (Carbonari et al., 2020). Sharkey et al. (2020) explain that glyphosate is also formulated as a salt, the inclusion of an amine or cation may have impacts on dicamba volatilization, in addition to the effects already reported for pH changes. These authors observed that the presence of glyphosate free acid in combination with dicamba and amines promoted high levels of volatility compared to those with only dicamba free acid.

Literature points out that the relationship of dicamba and pH is linked to degradation using various Fenton reactions (Huston, Pignatello 1999) or the effect of pH on sorption phenomena in several solid matrices such as clays since dicamba salt is an ionizable organic acid and its behavior is also affected by pH (Carrizosa et al. 2001).

The pH of the spray solution containing dicamba is an important factor in off-target movement, with solutions below pH 5.0 being associated with more off-target movement. Thus, one may suggest that dicamba volatility could be reduced by increasing the pH of the spray solution (Hemminghaus et al., 2017; MacInnes, 2017).

BAPMA dicamba salt is, in theory, more stable than the DGA salt, and undergoes less volatilization (Xu et al., 2012). The mixture of dicamba and glyphosate is considered to have a high volatility risk for dicamba salt and is more likely to undergo volatilization mainly because glyphosate substantially alters the pH of the spray solution, favoring the release of dicamba acid from the formulation by hydrolysis (MacInnes, 2017; Mueller, Steckel, 2019; Soltani et al., 2020). According to a study by Muller and Steckel (2019b), the pH of solutions containing a mixture of dicamba DGA and glyphosate was lower than or equal to 5 in all evaluations performed and ranged from 4.7 to 5. With the addition of a VRA to the mixture, its pH increased to around 6. Thus, glyphosate acts in the solution as a pH reducer and enhances volatilization of dicamba acid.

It is already known that diamine, solution and isopropylamine salts can influence the pH in the spray solution, as the pH of the solution can increase with dilution which contributes to the volatility of dicamba pH decreases more available hydrogen ions in the medium, allowing rapid formation of dicamba acid, which is more prone to volatilization. However, occasionally dicamba BAPMA salt with other glyphosate formulas also lowers the pH of the solution, which does not reflect the increase in plant injury, demonstrating that raising the pH may not increase the volatility of dicamba salt.

According to Muller and Steckel (2019b), the addition of BAPMA formulation increased the pH of the solution when the pH of the water source was below 5.75 but reduced the pH in more alkaline water sources. According to the authors, the addition of glyphosate potassium salt to the mixture of BAPMA dicamba formulation lowered the pH of the mixture by 1.5 to 1.9 units where BAPMA alone always had a pH > 6.2. When glyphosate potassium salt was added to BAPMA, the mixture mostly had a pH < 5.0, corroborating the results observed in Experiment III where the addition of glyphosate potassium salt lowered the pH of the mixture by 1.7 units.

Similar to the response of the BAPMA formulation both in the study by Muller and Steckel (2019b) and in the observations from Experiments I and II described herein, the addition of glyphosate potassium salt to the DGA formulation decreased the pH of the mixture to below 5.0. Thus, this pH drop may likely increase dicamba volatility. A possible explanation for this behavior is the cationic interaction in the spray mixture with the addition of ammonium salt and potassium salt that may increase the concentration of cations in the spray solution.

Various glyphosate formulations have different inert ingredients that could impart various attributes and affect dicamba volatility under field conditions. The mixture of dicamba DGA with VRA with lower volatility is believed to be related only to pH; therefore, the isopropylamine salt of glyphosate would be equivalent to the potassium salt in relation to the volatility of dicamba (Witten 2019).

Injury in Experiment I was reflected in plant height in all tested mixtures. The greatest loss in crop height at harvest was evident following treatment with dicamba and ammonium salt. In Experiment II, smaller heights were registered after treatment with ammonium salt. In Experiment III, the observed injury was reflected in the height of the plants at harvest, which reduced by almost 26% after treatment with the mixture of BAPMA and ammonium salt glyphosate.

According to Grossmann (2000) and Bunch et al. (2012), dicamba can promote cell elongation and differentiation and induce abnormalities in plant growth, epinasty, chlorosis, necrosis, and plant death through the destruction of membranes, vacuoles, and the vascular system. Wax et al. (1969) observed a greater increase in lateral branches from the node below the apical meristem that was compromised after application of dicamba in pre-flowering phase. Thus, the plants are able to quickly produce additional branches that can improve seed production per plant.

Plant branching proved to be little influenced by potassium salt (Experiment I) and more influenced by mixtures of BAMPA dicamba salt and ammonium salt, dimethylamine salt formulations, and glyphosate di-ammonium salt in Experiment III. This suggests a possible compensation of productive factors affected by the mixtures, such as plant height and crop injury, which was higher with the mixture containing ammonium and di-ammonium salt as previously observed.

Branching may represent a strategy to recover from the toxicity caused by herbicides and is possibly related to the death of the apical bud, extensively observed in this study. Bauerle (2014) also observed chlorosis of end leaves, cupping and wrinkles on upper leaves, swollen petiole base and leaf petiole, and stem epinasty, in addition to necrotic end points and detached end leaves. According to Balbinoti (2018), the relative contribution of branches to grain yield is an important factor representing between 20% and 40% of the yield; the greater the contribution, the smaller is the plant population per square meter.

The authors Bauerle (2014) and Weidenhamer et al. (1989) reported the relationship between plant height decrease and grain yield. However, the volatilization of the products in Experiments I, II, and III did not affect the productive capacity of the crop even at concentrations higher than four times the recommended concentration, as evident from the data on the weight of grains per plant. This result corroborates with the result reported by Bauerle (2014) who points out that the potential for dicamba injury at low concentrations, representative of the volatility concentrations, would not have a great effect on soybean yield, with the main concerns being the movement of the herbicide to sensitive areas owing to the contamination of the spray tank and drifts from the spray device.

The use of dicamba requires caution owing to different possibilities of the herbicide moving in the treated areas. When dicamba is not absorbed by soybeans or weeds, the herbicide present on the surface of the leaves can be rehydrated by dew at night and converted into dicamba acid, which has a high potential for volatilization. According to Jones et al. (2019), if the application of dicamba is postponed to later stages, when there is more foliage, it can enhance the secondary movement because the contact surface between the product and surface is increased; this would increase the availability of dicamba acid, which is susceptible to volatilization.

Other factors can influence the volatilization of dicamba, such as temperature and relative humidity. Behrens and Lueschen (1979) reported that an increase in temperature from 20°C to 30°C doubled the response of soybeans to dicamba dimethylamine salt in experiments performed in closed chambers. Furthermore, reduction in the relative humidity from 70% to 75% led to an increase in the soybean response to the volatility of dicamba salt dimethylamine.

Despite several modifications already made in dicamba herbicide formulations to reduce its potential risk of volatilization, Jones et al. (2019) and Norsworthy et al. (2018) reported that these new formulations still have the potential to volatilize to untreated areas under certain environmental conditions even when applied according to manufacturers' recommendations.

The off-target movement of a pesticide involves several factors such as spray equipment, wind speed, crop stage and sensitivity, atmospheric conditions, and spray solution properties (Lofstrom et al., 2013; Felix et al., 2014; Bish, Bradley, 2017). In this study, the low tunnels served to concentrate the movement of the products from each treatment, especially by vaporizing the product beyond the boxes where they had been applied.

The present study observed that the dicamba off-target movement due to volatility can expose susceptible plants to the herbicide. The addition of VRA to mixtures of glyphosate and dicamba is an important strategy to reduce the risks of dicamba volatilization. The mixture with glyphosate in the potassium salt formulation proved to be more adequate. It is also recommended to apply dicamba without mixing it with glyphosate. Mixing dicamba with ammonium salt of glyphosate is not recommended either.

REFERENCES

Anonymous (2018a) Engenia label. http://www.cdms.net/ldat/ldDG8028.pdf. Access: August 23, 2019

Anonymous (2018b) XtendiMax label. http://www.cdms.net/ldat/ldDF9006.pdf. Access: August 23, 2019

Anonymous (2020) XtendiMax with VaporGrip Technology Herbicide Product Label. EPA Registration No. 264-1210. 2020.

Behrens R, Lueschen WE (1979) Dicamba volatility. Weed Sci 27:486–493

Bish MD, Bradley KW (2017) Survey of Missouri pesticide applicator practices, knowledge, and perceptions. Weed Technology 31:165–177

Busey P, Broschat TK, Johnston DL (2003) Injury to landscape and vegetable plants by volatile turf herbicides. Horttechnol 13:650–652

Burke IC, Troxle SC, Askew SD, Wilcut JW, Smith WD (2005) Weed management systems in glyphosate-resistant cotton. Weed Technol 19:422–429

Carbonari, CA, Costa, RN, Bevilaqua, NC, Pereira, VGC, Giovanelli, BF, Lopez Ovejero, RF, Palhano, M, Barbosa, H, Velini, ED (2020) Volatilization of standalone dicamba and dicamba plus glyphosate as function of volatility reducer and different surfaces. Agriculture 10:495

Carrizosa, MJ, Koskinen, WC, Hermosin, MC, Cornejo, J (2001) Dicamba and sorption desorption on organo-clays. Applied Clay Sci 18:223–231

Corbett JL, Askew SD, Thomas WE, Wilcut JW (2004) Weed efficacy evaluations for bromoxynil, glufosinate, glyphosate, pyrithiobac, and sulfosate. Weed Technol 18:443–453

Culpepper AS (2006) Glyphosate-induced weed shifts. Weed Technol 20:277–281

Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. Environ Toxicol Chem 31:1023–1031

Hager A (2017) Observations of Midwest weed extension scientists. Page 98 in Proceedings of the 72nd Annual Meeting of the North Central Weed Science Society. St. Louis, MO: North Central Weed Science Society

Heap I (2020) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Access: February 8, 2020

Hemminghaus JW, MacInnes A, Zhang J, Inventors Monsanto Technology Llc, Assignee (2017) Low volatility herbicidal compositions. US Patent 9, 743, 664 B2

Huston, PL, Pignatello, JJ (1999) Degradation of selected pesticide active ingredients in commercial formulation in water by photo assisted Fenton reaction. Water Res 33:1238–1246

Johnson VA, Fisher LR, Jordan DL, Edmisten KE, Stewart AM, York AC (2012) Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2, 4-D. Weed Technol 26:195–206

Jones GT (2018) Evaluation of Dicamba Off-Target Movement and Subsequent Effects on Soybean Offspring. 197f. Thesis (Master of Science in Crop, Soil & Environmental Sciences) - University of Arkansas, Fayetteville

Jones GT, Norsworthy JK, Barber T, Gbur E, Kruger GR (2019) Off-target movement of DGA and BAPMA dicamba to sensitive soybean. Weed Technol 33:51–65

Kruger GR, Davis VM, Weller SC, Johnson WG (2010) Control of horseweed (Conyza canadensis) with growth regulator herbicids. Weed Sci 24:425–429

MacInnes A (2017) VaporGrip® technology: how it works and its benefits. Abstracts of the Weed Science Society of America 45:160. Westminster, CO: Weed Science Society of America

Meyer CJ, Norsworthy JK, Young BG, Steckel LE, Bradley KW, Johnson WG, Loux MM, Davis VM, Kruger GR, Bararpour MT, Ikley JT, Spaunhorst DJ, Butts TR (2015) Herbicide program approaches for managing glyphosateresistant Palmer amaranth (Amaranthus palmeri) and waterhemp (Amaranthus tuberculatus and Amaranthus rudis) in future soybean-trait technologies. Weed Technol 29:716–729

Meyer CJ, Norsworthy JK, Kruger GR, Barber LT (2016) Effect of nozzle selection and spray volume on droplet size and efficacy of Engenia tankmix combinations. Weed Technol 30:377–390

Mueller TC, Wright DR, Remund KM (2013) Effect of formulation and application time of day on detecting dicamba in the air under field conditions. Weed Sci 61:586–593

Mueller, TC (2015) Methods to measure herbicide volatility. Weed Sci 63116-120

Mueller TC, Steckel LE (2019a) Dicamba volatility in humidomes as affected by temperature and herbicide treatment. Weed Technol 33:541–546

Mueller TC, Steckel L (2019b) Spray mixture pH as affected by dicamba, glyphosate and spray additives. Weed Technol 33:547-554

Monsanto Brasil. Xtendicam®. Available online: https://plataformaintacta2xtend.com.br/ Access: May 18, 2022

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31–62 (Special Issue)

Sharkey, SM, Stein, A, Parker, KM (2020) Hydrogen bonding site number predicts dicamba volatilization from amine Salts. Environ. Sci. Technol 54:13630–13637

Silva DRO, Silva EDN, Aguiar ACM, Novello BD, Silva AAA, Basso CJ (2018) Drift of 2,4-D and dicamba applied to soybean at vegetative and reproductive growth stage. Cienc. Rural 48:1-7

Sciumbato AS, Chandler JM, Senseman SA, Bovey RW, Smith KL (2004) Determining exposure to auxin-like herbicides. II. Practical application to quantify volatility. Weed Technol 18:1135–1142

Steckel LE (2007) The dioecious Amaranthus spp.: here to stay. Weed Technol 21:567–570

Strachan SD, Casini MS, Heldreth KM, Scocas JA, Nissen SJ, Bukun B, Lindenmayer RB, Shaner DL, Westra P, Brunk G (2010) Vapor movement of synthetic auxin herbicides: Aminocyclopyrachlor, aminocyclopyrachlormethyl ester, dicamba, and aminopyralid. Weed Sci 58:103–108

Strachan, SD, Ferry, NM, Cooper, TL (2013) Vapor movement of aminocyclopyrachlor, aminopyralid, and dicamba in the field. Weed Technol 27:143-155

Striegel, S, Oliveira, M, Arneson, N, Conley, S, Stoltenberg, D, Werle R (2021) Spray solution pH and soybean injury as influenced by syntetic auxin formulation and spray additives. Weed Technol 35:113-127

Vangessel MJ (2001) Glyphosate-resistant horseweed from Delaware. Weed Sci 49:703–705

Werle R, Oliverira MC, Jhala AJ, Procotor CA, Rees J, Klein R (2018) Survey of Nebraska farmer’s adoption of dicamba-resistant soybean technology and dicamba off-target movement. Weed Technol 32:754–761

Witten T (2019) Understanding spray solution pH with Xtendimax with VaporGrip Technology. https://monsanto.com/app/uploads/2021/03/Understanding-Spray-Solution-pH-with-XtendiMax.pdf (Available from the author)

FINAL CONSIDERATIONS

Low concentrations of dicamba herbicide promote lesions on non-tolerant soybean (*Glycine max*) plants, even in concentration below 2 g ea ha^{-1}. The effect of low concentrations of dicamba in soy and yield depend on the phenological stage of the plant at the time of exposure. The volatility of dicamba can be managed through the use of volatility reducers and the correct formulation of the products used in the mixtures. Glyphosate potassium salt proved to be the safest choice to combine with dicamba salt without increasing the volatility relative to dicamba alone. The addition of a volatility reducer was efficient in reducing volatility for dicamba alone and DGA in combination with all glyphosate salts. One association with lower volatility was observed for dicamba with glyphosate potassium salt and the volatility reducer. This research showed that non-technical use of dicamba in Brazil could cause losses in neighboring crops due to its off-target transport, with significant potential for damage, as discussed in this study. Inspection and environmental responsibility are fundamental for the good use of this tool, as well as, more studies are necessary to subsidize the best position of this product within the production system.

REFERENCES

Benbrook CM (2016) Trends in glyphosate herbicide use in the United States and globally. Env Sci Eur 28:3.

Bunch, TR, Gervais, JA (2012). Dicamba Technical Fact Sheet.

Duke SO (2012) Why have no new herbicide modes of action appeared in recent years? Pest Manag Sci 68:505–512.

Egan, JF, Mortensen, DA (2012). Quantifying Vapor Drift of Dicamba Herbicides Applied to Soybean. Environmental Toxicology and Chemistry*, 31*(5), 1023--1031. EPA, U. S. (2006). *Reregistration Eligibility Decision for Dicamba and Associated Salts*

EPA - United States Department of Agriculture - National Agricultural Statistics Service (2016) NASS Quick Stats. http://quickstats.nass.usda.gov/. Accessed September 22, 2016

Heap, International Survey of Herbicide Resistant Weeds. 2018. Disponível em: www.wssa.org

James, C. Global status of Commercialized biotech/GM Crops: 2012. ISAAA Briefs 44. Disponível em: https://www.cabdirect.org/cabdirect/abstract/20113086248. Acesso em 21 de mar de 2012.

Norsworthy, JK et al. Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Science, v.60, p.31–62, 2012.

Oerke, EC Crop losses to pests. Journal of Agricultural Sciences, v.144, p.31-41, 2006.

Peterson, MA et al. 2,4-D past, present and future: A review. Weed Technology, v.30, p.303-345, 2016.

Silva, OC Tecnologia de aplicação de fungicidas. In: Canteri, MG, Pria, MD, Silva, OC (Eds.) Principais doenças fúngicas do feijoeiro. Ponta Grossa: UEPG, p.127-137, 1999.

Tan S, Evans RR, Dahmer ML, Singh BK, Shaner DL (2005) Imidazolinonetolerant crops: history, current status and future. Pest Management Science v.61, p.246–257.