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Dicamba tank mixtures and formulations and their effects on sensitive crops during cleanout procedures

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DICAMBA TANK MIXTURES AND FORMULATIONS AND THEIR EFFECTS ON SENSITIVE CROPS DURING CLEANOUT PROCEDURES

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DRIFT REDUCING ADJUVANTS, CLETHODIM AND THEIR IMPACT ON DICAMBA CLEANOUT PROCEDURE

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The introduction of dicamba-tolerant (DT) soybeans (*Glycine max L. Merr*) and cotton (*Gossypium hirsutum L)* in 2017 provided an additional tool for herbicide resistant weeds management. In the subsequent years, off-target movement of dicamba allegedly caused damage to sensitive crops and vegetation.

Possible causes of off-target movement include tank contamination, physical drift, and volatility. Additional products, such as herbicides to control grass, are often added to tank with dicamba, which is used to control broadleaf weeds, to increase the spectrum of control and application efficiency. Dicamba products registered for DT crops require the use of drift reducing agents to mitigate unintended effects to adjacent crops.

Sprayers are complex machines with valves, hoses, tanks, and nozzles that can retain herbicide residues and cause symptomology and/or injury to crops if proper cleanout procedures are not performed. Recommended cleanout procedures can be found in dicamba product labels, but there is no information available reporting the effect of tank mixtures or different dicamba formulations on retention of residues.

The objective of this research was to: 1) evaluate the dicamba retention of potential tank mixtures with dicamba and drift reducing adjuvants, clethodim as well as tank-cleaning agents on non-DT soybeans, 2) evaluate the cleanout procedures of commonly used dicamba products, on non-DT soybean, and 3) investigate how the rinsate following cleanout procedures of dicamba mixtures affect such as soybean, cotton, tomatoes (*Lycopersicon esculentum*) and peanuts (*Arachis hypogaea L.*).

Keywords: Sprayer cleanout, formulation, dicamba injury, yield, rinse

Dedication

To family and friends.

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Chapter 1

Literature review

Weed control is considered one of the most impactful factors influencing yield. Weeds can reduce soybean (*Glycine max L. Merr)* yield up to 52% when no weed management practices are implemented (Soltani et al. 2017). Glyphosate-resistant soybeans were introduced in 1996 and this was the first of several glyphosate resistant crops, allowing this herbicide to be sprayed over the top as a post-emergence herbicide (Duke 2014). The presence of acetolactate synthase (ALS) inhibitor resistant and glyphosate-resistant weeds challenged farmers even more, lessening the effectiveness of glyphosate and ALS-inihibitor based weed management systems (Wise et al. 2009; Culpepper et al. 2006).

Weed scientists and chemical companies have been developing new strategies and products to help producers better manage herbicide resistant weeds, including the use of crop rotation, pre-emergent herbicides, and tank-mixtures utilizing multiple herbicides sites of action. Combining multiple effective active ingredients, reduces the selection pressure and delays the evolution of herbicide-resistant weeds (Jhala et al. 2013; Ganie et al. 2017).

The selection of an herbicide-resistant trait in crops should be considered as part of the weed management program. The adoption of glyphosate-resistant crops and the repeated use of the same herbicide program can select individual plants that have some tolerance or resistance and shift the population for a resistant population (Owen et al. 2005).

In response to the growing concerns of weed resistance, dicamba-resistant soybean and cotton traits were released to the market in 2017, as an attempt to provide growers another herbicide-resistant management tool (Martin et al. 2006; Kniss 2018; EPA 2017).

Dicamba is an auxinic herbicide belonging to the plant growth regulator mode of action and was first described in 1958 and registered for use in 1962 (Hartzler 2017). Currently there are five synthetic auxin sites of action: phenoxy carboxylic acids, pyrydinecarboxylic acids, aromatic carboxymethyl, benzoic acids and quinolinecarboxylic acids. These sites of action are used in agriculture as herbicides in pre-emergence or post emergence weed control programs in a variety of crops. Some processes are affected by natural auxins such as cell elongation, cell differentiation, cell division, leaf senescence and tropic response (Grossman 2009).

High doses of synthetic auxins are believed to alter cell wall plasticity and nucleic acid metabolism. Increasing the pH of cell walls results in their elongation by increasing the activity of enzymes responsible for loosening the cell wall. This unbalance leads to uncontrolled growth of cells and eventually destruction of vascular tissue. High concentrations of auxins can cause plant overproduction of ethylene, culminating in an epinastic response and eventual death. However, in low concentrations auxins can increase RNA, DNA and protein biosynthesis through the stimulation of the RNA polymerase (Song 2014).

Dicamba was commonly used on non-crop areas and monocot crops, which are able to metabolize this herbicide and prevent injury (Chang and Born

1971), however, transgenic cultivars have granted a wider use pattern (Mortensen et al. 2012).

Low doses of dicamba are capable of inducing plant injury responses easily distinguished from other herbicide modes of action and can appear just hours after exposure. The most obvious symptoms are twisting or epinasty of stems and cupping of leaves (Egan et al. 2014).

An increase in reports of susceptible crop injury caused by off-target movement of dicamba occurred increased since the introduction of resistant cultivars in cotton and soybeans (EPA 2017). A total of 1.46 million hectares were reportedly affected by off-target movement of dicamba in 2017, and in 2018 the number decreased to 445 thousand hectares (Bradley 2017, Bradley 2018). Despite the reduced area in the following year, off-target movement remained a concern for this herbicide, especially regarding specialty crops and non-resistant cultivars.

Dicamba injury symptoms and yield loses caused by regular rates or sub rates are well documented in the literature in several different crops such as snap bean, sweet potatoes, strawberry, watermelon, grapevines, pecan tree, apple, raspberry, peach trees, and others (Colquhoun et al. 2017; Dintelmann et al. 2020; Culpepper et al. 2018; Shankle et al. 2021).

Kruger et al. (2012) reported dicamba exposure can be detrimental to tomato production, especially in early blooming. High doses of dicamba caused abortion of flowers, affected fruit maturity and weight. Comparable findings were reported by Jordan and Romanowski (1974), in which dicamba and 2,4-D caused similar symptoms on tomato plants, but a greater yield loss was reported with dicamba than compared to the same rate of 2,4-D.

Leon et al. (2017) used dicamba in peanuts at 10, 20 and 30 days after planting, and post emergence in three different growth stages (V2, V3 and V5) and five application rates of 35, 70, 140, 280 and 560 g ae ha^{-1}. The author concluded sensitivity to dicamba increased as plants approach the reproductive stage and the injury also increases as the rates increases, with yield reduction reaching up to 88 to 95% when sprayed at V3 and V5, respectively.

Growth regulators damage in cotton was first documented after 2,4-D became commercially available (Staten 1946). Smith et al. (2010) observed yield reductions from both 2,4-D and dicamba. Hamilton and Arle (1979) reported dicamba applied over the top of sensitive cotton before bloom decreases the cotton foliage, yield, boll components and fiber properties more than when applied later in the season.

The authors Soloman and Bradley (2014) sprayed soybeans with dicamba at 0.028, 0.28, 2.8 and 28 g ae ha^{-1} in two stages (V3 and R2). Symptoms diminished from 14 to 28 days after application when plants were sprayed at either stage, however, yield loss only occurred when sprayed at R2. Griffin et al. (2013) reported soybeans are 2.5 times more sensitive to dicamba injury at R1 than V2-3 stage. Osipitan et al. (2019) sprayed 6 micro rates of dicamba on soybeans during V7/R1 and concluded 1/1750 of the label rate can reduce yield in 10%.

The severity of crop damage is directly related to growth stage and amount of active ingredient carried by the off-target movement (Kelley et al. 2005, Andersen et al. 2004). Dosages as low as 0.028 g ha^{-1} are reported to cause visual symptomology on non-DT soybeans (Soloman and Bradley 2014; Kelley and Riechers 2007), yet yield is not affected until doses of 0.15 g ae ha^{-1} (Kniss 2018). Soybean plant height may be used as an indicator of yield reduction caused by dicamba (Weidenhamer et al. 1989). The presence of visual injury with exposure to low doses, although not necessarily resulting in an economic impact, has caused concern regarding the safety of dicamba; and thus amplified the need for mitigation of off-target movement prevention strategies.

Dicamba is no more susceptible to drift then other herbicides, however due to its low quantity to cause visual injuries on soybeans, it can be thought to be more harmful than other active ingredients. Mitigation practices of off-target movements should be adopted to reduce the risk of damaging sensitive crops in adjacent fields.

Spray particle drift is one cause of off-target movement of dicamba. Spray particle drift is impacted by several factors. An increase in wind speed, application pressure and boom height are correlated in an increase in downwind spray deposition in field situations (Nordby and Skuterud, 1975). Particle drift decreases with downwind distance, but it can cause 1% of visual estimation of injury at 293 meters from the edge of the sprayed field (Soltani et al. 2020). Previous field and laboratory studies have shown droplet size can be manipulated by nozzle selection and use of adjuvants (Alves et al. 2017). The selection of the correct adjuvant can significantly reduce drift, and inclusion in tank-mixtures is mandatory for some dicamba labels. (Johnson 2006, Oliveira et al. 2013).

Applications performed under high temperatures and low relative humidity can cause the release of dicamba vapors, resulting in injury to nearby susceptible fields (Egan and Mortensen 2012). Recent formulations include an amine salt known as BAPMA, and diglycolamine salt with VaporGrip Technology that increase formulation stability when exposed to adverse environmental conditions, reducing the volatilization risk (Abraham 2018). Bish et al. (2019) testing these new formulations reported that dicamba concentration in the air decreases during time but was still present in the air after 72 hours after treatment, being a possible source of injury especially when combined with glyphosate. Taylor (2021) reported that solutions containing vapor reducing adjuvants reduce soybean injury when compared to dicamba without this product. In the same study, Taylor reported that the combination of a drift reducing adjuvant and a vapor reducing adjuvant can be beneficial to reduce injury caused by volatility.

In a survey conducted by Werle et al. (2018) in the state of Nebraska respondents reported volatility from applications in dicamba-tolerant soybeans or corn were the main cause of injury, followed by physical particle drift, with 48% and 19% of responses, respectively. Tank contamination was identified as a source for dicamba injury by 6% of survey respondents, suggesting that those surveyed may be underestimating this as a source of off-target movement.

Inadequate cleaning of sprayer tanks, and the resulting contamination of subsequential applications, is a cause of synthetic auxins off-target movement (Boerboom, 2004). Although dicamba is considered highly water soluble at 6.5 g L^{-1} (Kamrin et al. 2010), it requires more time and effort to be removed when compared to glyphosate to a level that does not cause visual injury on soybeans (Steckel et al. 2010). It is important to consider the complexity of sprayer components, and the ease at which herbicide products may settle in various parts providing a source of contamination for future applications. Research conducted by Cundiff et al. (2017) shown that different agricultural hose can retain dicamba following cleanout procedures, where polyurethane blend and synthetic rubber retained the most residue when compared to a polyethylene blend.

Due to the potential for dicamba tank contamination, every dicamba label contains information regarding proper cleanout procedure. Directions vary by commercial product, but in general state to begin by emptying the tank of the primary solution before adding additional water. It is recommended to fill the tank with water up to 10% tank capacity and let the solution circulate for 15 minutes with valves open, and then flush liquid from the tank. This procedure should be repeated for a second rinse with the addition of a sprayer cleaner system to further break down pesticide residues, if indicated by the label. All strainers, screens and filters should then be scrubbed in a bucket with water and replaced. The third rinse consists of water alone to remove all the small particles left in the system and the sprayer system cleaner. Rinsates should be disposed in compliance with local, state, and federal legislation guidelines.

Tank cleaners can break down herbicide residues facilitating their removal by different mechanisms, including increasing solution pH and increase the solubility of weak acids. Others such and diesel fuel and kerosene can aid in the removal of oil-soluble herbicides such as 2,4-D. Ammonia penetrates and loosens deposits by raising the solubility of some pesticides, however it does not decompose them (Johnson et al. 1999; Pringnitz 1997).

Browne et al. (2020) performed cleaning procedures in 25 agricultural sprayers using water, glyphosate, and two commercial cleaners, Fimco™ (Fimco Industries, North Sioux City, SD 57049, USA) detergent and Protank® (Winfield Solutions, LLC, St. Paul, MN 55164, USA) detergent. Two samples were collected from each section of the sprayer after half of the solution was flushed. The study concluded that if a tank is triple rinsed and the minimum amount of water (≥ 10% of tank volume) is used, it is enough to avoid dicamba symptoms in sensitive crops using just water and no sprayer plumbing system cleaner. Boerboom (2004) conducted a study testing clean-out procedure for dicamba using water and ammonia for the second rinse in an 190-liter poly tank and detected residues in the spray tank and the boom at 0.024% and 0.63% of the dicamba label rate, respectively.

Purpose of Research

Due to the number of off-target movement reports in 2017 and 2018, dicamba injury is a primary concern in soybean and cotton production. Regulations have been introduced to mitigate dicamba off-target movement. In 2021, the use of a drift reducing agent became mandatory for certain dicamba products. Certain dicamba labels also require a plumbing system cleaner to be added in the second rinse, however, a couple of studies suggest that their use does not make any difference when comparing to using just water.

Five dicamba formulations currently exist in the market, but there is no data regarding the effect chemical formulation has on tank cleanout efficiency. Each formulation contains different concentrations of dicamba active ingredient and recommended label use rates. Literature states that a 0.028 g ha^{-1} of dicamba are capable of inducing injury to soybeans (Soloman and Bradley 2014), and dicamba remaining after tank cleanout can be a source of injury on sensitive crops, and unless applicators are capable of utilizing multiple sprayers for treatments containing non-dicamba solutions, the need for proper cleaning procedures is imperative.

The objective of this research was to: 1) evaluate injury on non-DT soybeans resulting from tank retention of dicamba following the employment of sprayer plumbing system cleaners or not when using a tank mixture of dicamba, drift reducing adjuvants, clethodim, using, 2) evaluate tank cleanout procedures for commonly used dicamba products, and their effect on non-DT soybeans, and 3) Evaluate in the greenhouse the injury resulting from tank retention of dicamba following the use the use or not of sprayer plumbing system cleaners and tank mixtures of drift reducing adjuvants, clethodim in other sensitive crops such as tomatoes, peanuts and soybean.

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Chapter 2

Response of non-dicamba soybeans to tank contaminations of dicamba tank mixtures of clethodim, and drift reducing adjuvants.

Abstract

Herbicide resistance is a challenge for row crop producers. The development of dicamba-resistant soybeans and cotton broadened the spectrum of post-emergence herbicides options for managing of herbicide resistance. Coupled with the adoption of DT crops was the increased use pf dicamba and the increase reports of off-target movement of dicamba. Sprayer tank contamination is identified as one cause of off-target injury of dicamba. Injury caused by inadequate cleaning of sprayer tanks can range from minor visual symptoms with no yield impact to total yield loss and is directly related to growth stage of soybean at off-target application, and the amount of active ingredient residue in the sprayer.

The objective of this study was to evaluate injury on non-DT soybeans resulting from tank retention of following the use or not of sprayer plumbing system cleaners when using a tank mixture of dicamba, clethodim and drift reducing adjuvants. Tanks were rinsed four times, with a sample collected after each rinse to simulate a triple rinse procedure and a subsequent application. Rinsate solutions were sprayed on non-DT soybean at the R1 growth stage. Results indicate rinse number being a significant factor, as visual injury was observed following the first three rinses but not for the subsequent application. Plant height and yield increased with each rinse. Plants showed visual symptoms at the third rinse but no yield or height reduction occurred when compared to a non-treated check. If triple rinse procedures are followed accurately using the minimum water volume

recommended (≥ 10% of tank volume), visual injury should not be present in the follow-up application and the use of sprayer plumbing system cleaner is not necessary.

Introduction

The spread of glyphosate resistant weeds has been a challenge for farmers worldwide. Recent development of dicamba-tolerant soybeans and cotton provided growers in the US and Brazil a new tool for controlling herbicide resistant broadleaf weeds. The rapid adoption of dicamba-tolerant soybeans by North American growers is also expected to occur in some regions of Brazil due to the presence of herbicide resistant weeds. Oliveira et al. (2020) conducted a survey with Brazilian corn and soybean growers reporting 60% of participants intend to use a synthetic auxin trait in their future weed management programs.

Combining multiple sites of action in a tank mixture with dicamba can broaden the control of several weed species, reduce selection pressure for resistance impeding the evolution of herbicide-resistant weeds. (Ganie et al. 2017; Zimmer et al. 2018). Osipe et al. (2017) sprayed dicamba and 2,4-D with and without glyphosate and concluded the addition of another herbicide site of action can result in a synergistic interaction increasing weed control, even in glyphosate resistant populations.

The incorporation of dicamba-tolerant soybean varieties provides an additional tool for the management of resistant weeds. However, rapid adoption of the new technology without proper mitigation of off-target movement, led to a high rate of off-target movement complaints in the United States (Bradley 2017). This was likely due in-part to the fact that low doses of dicamba, a synthetic auxin herbicide, can cause injury several sensitive crops and vegetation. Doses as low as 0.028 g ha^{-1} can cause injury on non-DT soybeans and yield can be affected by doses of 0.15g ae ha^{-1} and higher (Kniss, 2018).

Solomon and Bradley (2014) sprayed low doses of dicamba (0.028, 0.28, 2.8 and 28 g ae ha^{-1}) at two soybean growth stages (V3 and R2) and although symptoms increased according to rate, no yield loss occurred in V3 stage at any dose. Yield loss was expressed in the two highest doses at the R2 application, despite no difference in visual estimation of injury as compared to the V3 timing.

The most common causes of dicamba off-target movement are spray particle drift, volatilization, and sprayer tank contamination. (Soltani et al. 2020; Behrens and Leuschen 1979; Boerboom 2004)

Particle drift decreases with downwind distance yet can cause 1% visual estimation of injury at 293 meters from the edge of a sprayed field (Soltani et al. 2020). To mitigate this source of dicamba off-target movement, it is recommended to select the nozzle types, and manipulate the droplet size with the use of drift reducing adjuvants is recommended (Butler Ellis et al., 1997). In 2021, the addition of drift reducing adjuvants became mandatory when applying Xtendimax® (Bayer CropScience LP 800 N. Lindbergh Blvd. St. Louis, Missouri 63167) (Anonymous 2021).

When sprayed at high temperatures and low relative humidity, dicamba can volatize and vapors may move to sensitive crops, causing injury (Behrens and Lueschen 1979). Some dicamba products recommended for soybeans are required to add volatility reducing adjuvants and it is recommended to be sprayed in low temperatures with high relative humidity to avoid injury by volatility (Abraham 2018).

Of the three major off-target movement causes, tank contamination is likely the most preventable (Werle et al. 2018). Effective clean-out procedures, if followed, can reduce the concentration of dicamba to a level that will not cause injury on subsequent crops.

A sprayer is a complex machine consisting of several valves, hoses and connections that can trap residues. The solution trapped in these hard-to-reach components may contaminate the subsequent tank mixture and can cause dicamba injury of the next field application is on a dicamba sensitive crop. Cleanout instructions, including proper triple rinse procedures, can be found in every dicamba product label. Boerboom (2004) found 0.63% of the initial concentration of dicamba in the third rinse using water and ammonia. After performing a triple rinse in 25 sprayers using different sprayer plumbing system cleaners and water, Browne (2020) concluded using the minimum amount of water (10% of tank volume) was enough to remove dicamba residues, without the use of a tank cleaner.

The objective of this study was to evaluate injury on non-DT soybeans resulting from tank contamination following the use or not of sprayer plumbing system cleaners when using a tank mixture of dicamba, clethodim and drift reducing adjuvants. The hypotheses of this study were: 1) dicamba tank mixtures will retain more dicamba during cleanout procedures; 2) triple rinse tank cleanout procedure will reduce dicamba concentration to a safe amount that will not cause dicamba symptoms in the follow up application; 3) the use of tank plumbing cleaner will help reduce dicamba on the third and follow-up rinses.

Materials and Methods

Field experiments were conducted in the 2020 and 2021 growing seasons to evaluate crop response to rinsates following dicamba and clethodim tank-mixtures. The 2020 study was located at a commercial area near Stapleton, Nebraska, and the 2021 studies were conducted at the West Central Research Education and Extension Center in North Platte, Nebraska, the Havelock Research Farm in Lincoln, Nebraska. The study was conducted as a Row-Column block design and each treatment had four replications. Each experimental unit was 76 cm wide by 9.1 m in length and included four 30 cm space soybean rows. Non-DT soybeans were used in all locations, with planting parameters specified to each location (Table 1).

Experimental factors consisted of tank-mixture and tank rinse procedure. A treatment of water alone was included at each location as a treated control for further comparison. Treatment factors were single product and tank mixtures of XtendiMax® (Bayer CropScience LPP.O. Box 12014, 2 T.W. Alexander DriveResearch Triangle Park, NC 27709) at 1120 g ae ha^{-1} alone and in combination with either Intact® (5 ml L^{-1}) (1429 S. Shields Drive Waukegan, IL 60085), Trap Line Pro II® (5 ml L^{-1}) (CHS Inc. 5500 Cenex Drive Inver Grove Heights, MN 5507), or Select Max® (Valent USA Corporation, Walnut Creek, CA, 94596) at 136 g ae ha^{-1}. and a triple rinse cleanout procedure using water either with or without the addition of WipeOut XS® (5 ml L^{-1}) during the second rinse. A fourth rinse with water was collected to simulate a subsequent application. Samples were collected from each rinse cycle for all tank-mix and cleanout procedure treatments totaling 52 treatments and one check for each rinse.

The system used to simulate tank mix and cleanout procedure (Figure 1) consisted of four 189 L cone-bottom polyethylene tanks equipped with a Banjo polypropylene fitting (Banjo Corporation, Crawfordsville, IN) and a valve to ensure full drainage. Each tank mixture was recirculated through a diaphragm pump Shurflo® 2088-394-154 (Pentair, Minneapolis, MN) with an output of 12.1 L per minute and a 102 cm long synthetic rubber return hose was attached to an irrigation nozzle number 21 to equally distribute the water in the inner tank walls and ensure adequate residue removal.

To conduct the experiments, each of the four tanks were first filled with 113 L of water, and tank mixture products were added in the appropriate order. The solution circulated through the system for 20 minutes and then transferred to a separate chemical reservoir for disposal. After the tanks were emptied, 11.3 L of water with our without tank cleaner was added (10% of the initial tank volume) and circulated for 15 minutes when samples were collected and the remaining tank solution was transferred for disposal. Following all rinse procedure treatments, a fourth sample was obtained by filling to the tank mix volume (113 L) with water circulating for one minute before sample collection. A 60 ml sample was collected from each rinse for laboratory analysis, and four litters of solution from each rinse were collected for soybean response in field. Water used to simulate contamination and cleaning procedures were sourced from tap water with a temperature around 13°C.

Treatment application

To test the effect of the tank mixtures and their rinses on non-DT soybeans, applications were made with a CO_2 backpack sprayer, a 2m boom using AIXR10003VP nozzles (TeeJet Technologies, Springfield, IL) on 50 cm nozzle spacing and calibrated to deliver 140 L ha^{-1} at 138 kPa at ground speed of 6.3 km h^{-1}. Soybeans were at R1 growth stage when treatments were applied to each experimental unit. Samples from the follow-up applications were the first ones to be sprayed and the first rinse was the last sprayed to prevent any contamination building up in the spray equipment

Data Collection

Data were collected from the center two rows of each experimental unit. Four plants from each plot were arbitrarily selected and measured to determine plant height at 14 and 28 days after treatment. Visual estimations of soybean injury were collected 7, 14, 21 and 28 days after application on a 0 to 100% scale, with 0 representing no visible injury was visible and 100 representing total plant death. Soybeans were harvested from the center two rows of each experimental unit using a plot combine and yield was adjusted to 13% moisture. Three plants from each plot were arbitrarily removed prior to harvest and number of pods, number of seeds and seed weight data were evaluated. Samples collected from each rinse were analyzed with high performance liquid chromatography (HPLC) at the Mississippi State Chemical Laboratory to determine dicamba concentration.

Data were subjected to analysis of variance (ANOVA) to test significance of treatment effects using SAS (Statistical Analysis Software, version 9.4, Cary, NC, USA), all comparisons were performed at $\alpha = 0.05$ significance using a Fisher's Protected LSD test. Tank mixture, cleanout procedure and rinse number were considered fixed effects and location was considered a random effect.

Results and Discussion

Rinse was a main effect in all parameters evaluated, no main effect of tank mixture or interaction between mixture and rinse was significant for visual injuries in any of the evaluations performed.

The HPLC analysis show that there is no different between the solutions tested and the only effect significant was rinse, suggesting that the presence of the soap did not increase dicamba removal from the contaminated tanks (Table 2 and Table 3). The first rinse reported 45.6 g ha^{-1}, second rinse 3.4 g ha^{-1}, third rinse 0.36 g ha^{-1} and follow up 0.01 g ha^{-1}. Similar findings were reported by Browne et al. (2020), where no difference was noticed when using ammonia, two commercially available tank cleaners, or water. Dicamba concentrations decrease every rinse and can still be registered in the follow up application, however, differences were seen just between the first rinse and the other rinses.

No visual estimation of injury was observed in any evaluations in the follow up application (Table 4). At seven days after treatment (DAT), the estimation of injury was 53% in the first rinse, and 34% in the second rinse (Table 5). Estimations of injury observed in the third rinse were 11%. At 14 DAT, visual estimations of injury were 67%, 38% and 13% for the first, second and third rinse respectively. The evaluation performed 21 days showed a similar trend of the previous evaluation where the injury decreases according to rinse and it is not registered on the follow up application, with the first rinse reporting 70% of injury second reporting 44% and third reporting 14%. At 28 DAT, the first rinse showed higher rates of visual injury with 76% on the first rinse, followed by the second rinse at 48%, and third rinse has shown 14% of visual estimation of injury.

Results shown for plant height reduction were also significant to rinse, tank mixture was not significant and there was no interaction between the main effects (Table 5). Plant height reduction compared to the treated control shown height increases for each subsequent rinse, and no difference was reported between the third rinse and follow up (Table 6). Plant height reduction at 14 DAT demonstrate a reduction of 42% in the first rinse and 21% in the second. At 28 DAT, plant high reduction of the first rinse was 53% and in the second rinse was 25%

Yield reduction has also shown to be significant just for rinse and no interaction was noticed between the main effects (Table 4). The yield in control plots averaged 3,727 Kg ha^{-1} and the reduction was greater at the first rinsate, with 93% reduction. The second rinse reduced yield 17% and the third and fourth rinse did not show yield reduction (Table 4). Weidenhamer et al. (1989) reported soybean height may be used as a quick indicator for yield reduction caused by dicamba. Visual estimation of injury does not always lead to yield loss, which is dependent on herbicide rate and growth stage at time of exposure (Osipitan et al., 2019).

The yield reduction in the first two rinses emphasizes the importance of proper spray tank cleaning following dicamba applications prior to subsequent applications to dicamba non-DT soybeans or sensitive crops. Increasing the quantity of water used in each rinse can lower the concentrations of dicamba and reduce potential injury, but if the minimum amount (10%) is used and procedures are performed correctly, contamination and future injury in the follow up application can be avoided (Carpenter et al 2019).

Conclusion

Data from this study indicate no difference in dicamba residue removal between tank mixtures containing combinations of drift reducing adjuvants or clethodim. The addition of a tank cleaner did not decrease the amount of dicamba residue, suggesting three rinses of water was sufficient to remove dicamba residues. Dicamba visual estimation of injury decrease according to rinse and although present in plants treated at the third rinse, yield was not affected. Plant height reduction was greater in the first two rinses, with no plant height reduction observed in the fourth rinse when comparing to the third rinse. HPLC data show difference between the first rinse and the others. Besides the second, third and follow up no being different in this variable, symptoms were seen on non-DT soybeans. Triple rinsing sprayer tanks using a minimum of 10% of the tank capacity with water has proven efficient for reducing the dicamba concentration to a level that will not cause injury in the tank mixtures tested.

List of Tables

Table 1. Year, location, varieties, and population class for each experiment

Year	Location	Variety	Population Seeds ha^{-1}
2020	Stapleton	Enlist Mycogen 209E	320,000
2021	North Platte	Syngenta S28-E3	320,000
2021	Lincoln	Syngenta S28-E3	320,000

Table 2. Impact of number of rinses on soybean yield components in three studies from a sprayer contaminated with different dicamba tank mixtures.

Fixed effects	Parts per million
	P-value
Rinse	<0.001
Mixture	0.988
Rinse*Mixture	1.000

Table 3. Impact of number of rinses on soybean yield components in three studies from a sprayer contaminated with different dicamba formulations.

Fixed effects		
Rinse	—— g ha^{-1} ——	
1	45.560	A
2	3.353	B
3	0.362	B
4	0.008	B

Table 4. P-values for main effects and interactions of rinse and tank mixture for visual estimation of injury and yield reduction from a contaminated sprayer with different dicamba tank mixtures across four replications in three studies.

	Visual estimation of injury				
	Days after treatment				Yield
Fixed effects	7	14	21	28	reduction
	P-value				
Rinse	<0.001	<0.001	<0.001	<0.001	<0.001
Mixture	0.490	0.070	0.257	0.421	0.095
Rinse*Mixture	0.130	0.248	0.759	0.136	0.464

Table 5. Impact of number of rinses on visual estimation of injury on soybeans at 7, 14, 21 and 28 days after treatment and yield reduction from a sprayer contaminated with different dicamba tank mixtures across four replications in three studies.

	Visual estimation of injury[a]				
	Days after treatment				Yield
Rinse	7	14	21	28	reduction
	%				
1	53 A	67 A	70 A	76 A	93 A
2	34 B	38 B	44 B	48 B	17 B
3	11 C	13 C	14 C	14 C	-2 C
4	0 D	0 D	0 D	0 D	-3 C

[a] Means within columns followed by a common letter are not significantly different according to Fisher's Protected LSD test (α=0.05)

Table 5. P-values for main effects and interactions of rinse and tank mixture for plant height 14 and 28 days after application from a contaminated sprayer with different dicamba tank mixtures across four replications in three studies.

	Plant height reduction	
	Days after treatment	
Fixed effects	14	28
	P-value	
Rinse	<0.001	<0.001
Mixture	0.637	0.777
Rinse*Mixture	0.712	0.981

Table 6. Impact of number of rinses on plant height reduction on soybeans at 14 and 28 days after treatment from a sprayer contaminated with different dicamba tank mixtures across four replications in three studies.

	Plant height reduction[a]			
	Days after treatment			
Rinse	14		28	
	%			
1	42	A	53	A
2	21	B	25	B
3	1	C	0	C
4	-3	C	-2	C

[a] Means within columns followed by a common letter are not significantly different according to Fisher’s Protected LSD test (α=0.05).

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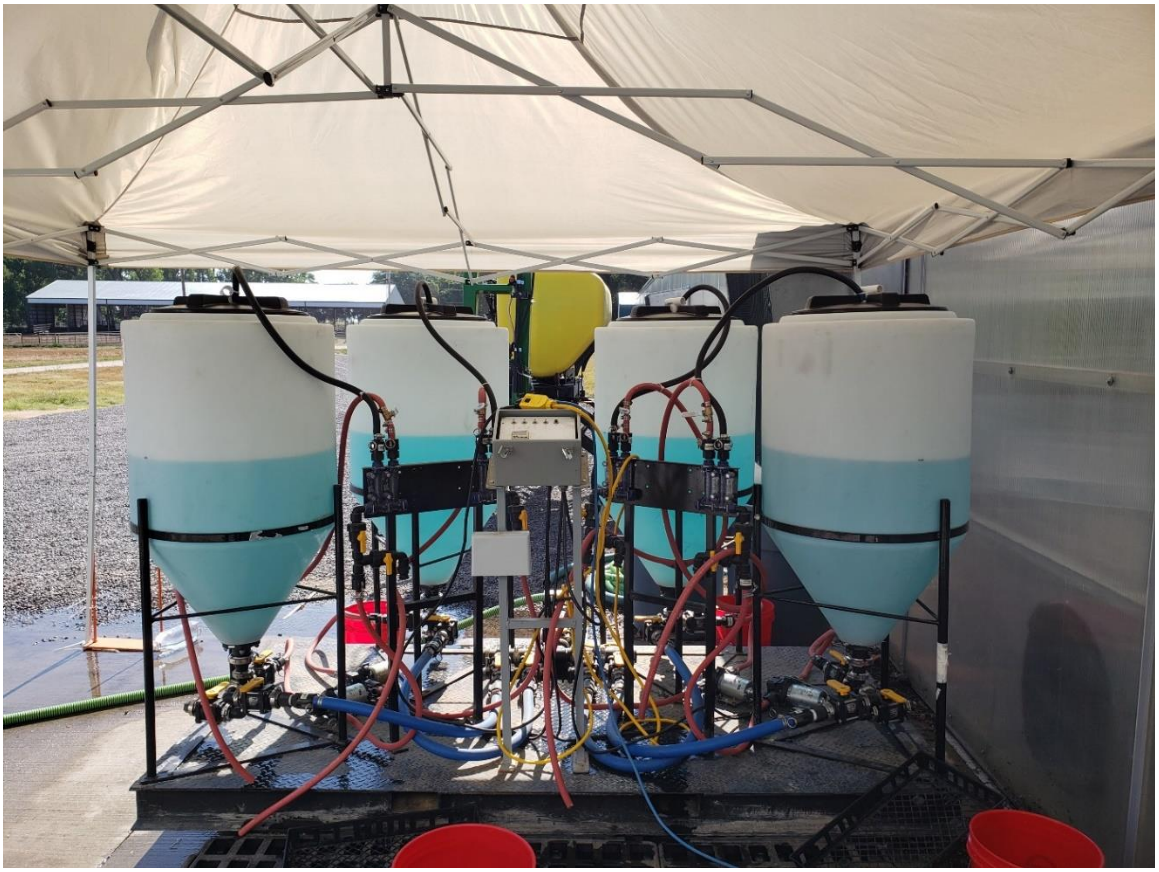

Figure 1. Small scale sprayer designed to conduct cleanout experiments.

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Chapter 3

Efficiency of tank cleaning different dicamba formulations

Abstract

The commercial introduction of dicamba-tolerant crops resulted in an increase in dicamba based herbicide use, and consequently an increase in dicamba off-target movement reports. Dosages as low as 0.028 g ae ha^{-1} can induce visual response on soybeans. Tank contamination has been identified as one source of off-target movement causing damage to sensitive crops or vegetation. There is little data regarding the effect of new dicamba products available to farmers on proper cleanout procedures. The objective of this study was to determine the effect of commercially available dicamba product rinsates on non-DT soybeans. Sprayer tanks containing dicamba products were rinsed to simulate a triple rinse procedure and a subsequent follow-up application, with rinsate samples collected after each rinse. Solutions were sprayed on non-DT soybeans at R1 growth stage. During this study, the main effect rinse was significant for all the parameters evaluated, no product effect or interaction between the main effects were noticed. Visual estimations of injury decrease each rinse and they were absent in the follow up application. Plant height and yield increases for each rinse and there were no differences in yield and plant height between the third rinse and follow up application, even though soybeans were injured in the third rinse.

Introduction

Soybeans and cotton cultivars with resistance to dicamba have are now commercially available. This new biotech allows dicamba to be sprayed over the top in these previously susceptible crops. For more than 50 years, dicamba has been used on corn, pasture, and small grains. Dicamba belongs to the benzoic acid family of the synthetic auxin group of herbicides. It is a relatively economical herbicide, with little risks imposed to humans, wildlife, or soil. (Mithila et al. 2011, Shaner 2014).

When used as a burndown or post application on monocot crops such as corn, dicamba can control glyphosate-resistant broadleaf weed populations, providing an important tool for managing herbicide resistance (Vink et al. 2013, Spaunhorst et al. 2014).

Pesticide applications occurring in extreme weather conditions, or with inadequate nozzle selection or solution can increase the potential of dicamba off-target movement (Alves et al. 2017; Byass & Lake, 1977; Egan and Mortensen 2012).

Leaf cupping, stem and leaf epinasty, cracked and swollen stems, and chlorosis followed by necrosis are the classic symptomology of reduced rates of dicamba off-target movement on sensitive crops (Kelley et al. 2015). In 2017, a total of 1.46 million hectares were reported with dicamba off-target movement (Bradley, 2017). Of the possible sources of off-target movement (particle drift, volatility, and tank contamination), tank contamination is the most preventable (Werle et al. 2018).

Synthetic auxins such as dicamba are known for their difficulty to be removed from the sprayer, despite a high solubility (6.5 g L^{-1}) in water (Boerboom 2004; Cundiff et al. 2017). Boerboom (2004) performed a sprayer cleanout using water and ammonia, and found dicamba in solution taken from the third rinse. The concentrations reported were low, but enough to cause injury. Osborne et al. (2015) conducted a cleanout survey concluding the amount of dicamba reduces from 241 μg mL^{-1} to 0.41 μg mL^{-1} after the third rinse using water. The concentration reduced exponentially as the number of rinses increased, with the first rinse responsible for removing 95% of the initial dicamba.

Carpenter (2019) evaluated sprayer cleanout using 10, 20, 40 and 60% of the total tank capacity with water and found rinsing with 10% was the minimum amount needed to properly remove dicamba residue and avoid plant injury in follow up applications.

Visual symptomology on sensitive plants can be observed with dicamba doses as low as 0.028 g ha^{-1}, and yield loss at doses of 0.15 g ha^{-1}. Yield loss is dependent on the stage of plants at the time of exposure. Soybeans in the vegetative stage are more likely to recover from the injury and do not suffer yield loss, as compared to those at reproductive stage (Soloman and Bradley 2014, Kniss 2018).

The addition of adjuvants, either included in the formulation or added separately, can affect the solution activity or spray characteristics (Penner, 2004). In-can adjuvants are those present in the formulation of the product and tank mix adjuvants are added to the tank by the applicator. The presence of adjuvants in the solution can modify droplet size, droplet evaporation, contact angle, surface tension, density, and viscosity (Xu et al. 2010, Cunha and Alves, 2009; Spanoghe et al. 2007, Bouse et al. 1990). There is little information regarding the effect in-can or tank mix adjuvants have on dicamba retention in sprayer systems.

The objective of this study was to evaluate the cleanout procedures of commonly used dicamba products on non-DT soybeans. The hypotheses of this study ware: 1) different dicamba formulations might influence retention on the sprayer; 2) soybeans will respond differently to each product used due to the different formulations; 3) triple rinsing the sprayer with water will reduce dicamba concentrations to an amount where the follow up application will not cause injury symptoms on non-DT soybeans.

Materials and Methods

Field experiments were conducted in 2020 and 2021 growing seasons to evaluate soybean response to rinsate solution from five different dicamba commercial products. The 2020 study was located at a commercial area in Stapleton, Nebraska, and 2021 studies conducted at the West Central Research, Education and Extension center at North Platte, Nebraska, Havelock Farm in Lincoln, Nebraska, and at the E.V Smith Research Center in Tallassee, Alabama. The study was conducted as Row-Column block design and each treatment had four replications. An experimental unit consisted of four rows of soybean with an area of 76cm by 9.1 m in length. Non-Dt soybeans were used in all locations, with planting parameter specified to each location (Table 1).

Experimental factors consisted of product and rinse. One treated control (water) was added to each location for further comparison. Treatments included: XtendiMax® (Bayer CropScience LPP.O. Box 12014, 2 T.W. Alexander DriveResearch Triangle Park, NC 27709) at 1120 g ae ha^{-1} , Diflexx® (Bayer CropScience LPP.O. Box 12014, 2 T.W. Alexander DriveResearch Triangle Park, NC 27709) at 560 g ae ha^{-1} , Enginea® (BASF Corporation 26 Davis Drive, Research Triangle Park, NC 27709) at 560 g ae ha^{-1}, Clarity® (BASF Corporation 26 Davis Drive, Research Triangle Park, NC 27709) at 560 g ae ha^{-1} and Status® (BASF Corporation 26 Davis Drive, Research Triangle Park, NC 27709) at 131 g ae ha^{-1}.

The system used to simulate tank cleanout (Figure 1) consisted of four 189 L cone-bottom tanks equipped with a Banjo polypropylene fitting (Banjo Corporation, Crawfordsville, IN) valve to ensure full drainage. Tank mixture was run through a diaphragm pump Shurflo® 2088-394-154 (Pentair, Minneapolis, MN) with an output of 12.1 L per minute and a 102 cm long synthetic rubber return hose was attached to an irrigation nozzle number 21 to equally distribute the water in the inner tank walls and ensure adequate residue removal.

Each tank was first filled with approximately 113 L of water, and tank mixture products next added in appropriate order. The solution circulated through the system for 20 minutes and disposed of in a chemical tank reservoir. After the tanks were emptied, water was added at 10% of the initial tank volume and allowed to circulate through the system for 15 minutes before sample collection and solution disposal for each rinse. A sprayer plumbing system cleaner was added during the second rinse of all treatments with the same collection parameters. The third rinse was collected using the same procedure as the first rinse and a follow-up was conducted by filling the tank to its initial volume (113 L) with water and allowing the solution to circulate for one minute before sample collection. With each rinse, a 60 ml sample was collected for future laboratory analysis, and four litters of solution collected for soybean response in field. Water used to simulate contamination and cleaning procedures were sourced from tap water with a temperature around 13°C.

Treatment application

Applications were made with a CO_2 backpack sprayer using AIXR10003VP nozzles (TeeJet Technologies, Springfield, IL) calibrated to deliver 140 L ha^{-1} at 138 kPa at ground speed of 6.3 km h^{-1}. Soybeans were at R1 growth stage and treatments were applied to the two center rows of each experimental unit, with the remaining two edge rows used as buffer rows. Samples from the follow-up applications were the first ones to be sprayed and the first rinse was the last sprayed to prevent any contamination building up in the spray equipment.

Data Collection

During the simulation of the contaminations, samples were collected from each rinse and analyzed with high performance liquid chromatography (HPLC) in the Mississippi State Chemical Laboratory to determine dicamba concentration. Field data were collected from the center two rows of each experimental unit. Four plants in each plot were arbitrarily selected for plant heights were made using a ruler at 14 and 28 days after treatment and compared to a sprayed check. Visual estimations of injury were collected 7, 14, 21 and 28 days after application on a 0 to 100% scale, with 0 being no injury visible and 100 being total plant death. Soybeans were harvested using a two-row plot combine and moisture was adjusted to 13% and percentage of yield reduction compared to the treated check were calculated. Prior to harvest, three plants per plot were arbitrarily removed to yield component analysis.

Data were subjected to analysis of variance (ANOVA) to test significance of the effects using PROC GLIMMIX on SAS (Statistical Analysis Software, version 9.4, Cary, NC, USA) all comparisons were performed at $\alpha = 0.05$ significance using a

Fisher's Protected LSD test. Product and rinse were considered fixed effects, and location was considered a random effect.

Results and discussion

The main effect, product, and the interaction between the two main factors were not significant in any of the parameter evaluated. Visual estimation of injury in applications decreased with each rinse and were not present in the fourth rinse, simulating a follow up application. Similar results were reported by Carpenter et al. (2019) where the cleaning sequence was not significant to reduce soybeans symptoms, but the number of rinses was.

Dicamba residue was reduced with each subsequent rinse, yet detectable in the follow up application. HPLC analysis shown rinse being significant, mixture and an interaction between the main effects in the first rinse (Table 2). XtendiMax® shown to have higher concentration than the other products and Status® has shown to have a lower concentration than the other in this rinse. On the subsequent rinses, no difference between the products were noticed (Table 3). Similar results were obtained by Marques et al. (2021) where each rinsate reduces the amount of dicamba on the tanks, but they are still present in the follow-up application and no visual symptoms were recorded.

Visual estimations of injury were significant to rinse, no interaction between the two main effects were noticed (Table 4). Visual estimation of injury at 7 DAT for the three rinses was 60%, 29% and 13%, respectively. At 14 DAT the injury increased to 60%, 35% and 16 for the first, second and third rinse. The first, second, and third rinse injury was 70%, 36%, and 16, respectively for 21 DAT. At 28 DAT, the injury increased again to 75%, 39%, and 20% for each rinse (Table 5). Similar findings were reported by Andersen et al. (2014), where symptoms decreased with lower doses of dicamba applied to soybeans.

The only significant main factor for plant height was rinse. Product and the interaction between the main factors were not significant (Table 6). Plant high reduction was greater at 28 DAT as compared to 14 DAT. Differences occurred between the first and second rinse, but not in the third and follow up application, despite visual estimations of injury were present in the third rinse. At 14 DAT, the reduction reported at the first rinse was 35 % and 14% in the second. The reduction at 28 DAT was 48 % and 19 % for the first and second rinse, respectively (Table 7). Marques et al. (2021) showed similar results when spraying dicamba sprayer contaminations, with the first rinse causing the highest reduction. No plant height reduction was observed in the second rinse of the same study due to higher water volumes added to each rinse, resulting in complete solubilizing of the dicamba.

Yield reduction followed the same trend, with yield increasing after each rinse with the only significant factor being rinse (Table 4). Yield in control plots was 4,700 Kg ha^{-1} and the first rinse reported the highest yield reduction with 83 %, followed by the second rinse with 14 %. The third and fourth rinses showed no yield reduction and were not different between them (Table 5). Comparable results were reported by Browne (2020), where triple rinsing with water alone reduced dicamba levels and prevented yield reduction.

Conclusion

Data from this study indicate no difference in dicamba residues using the different products tested, triple rinse using the tank cleaner has proven to be effective to reduce dicamba to a concentration that will not cause visual symptoms in soybeans, reduce plant height or reduce yield. HPLC data did not shown any difference between the products tested but has shown significant decreases in each consecutive rinse. Visual estimation of injury on the non-DT soybeans decreased each rinse, and even though they were noticed in the third rinse, it did not cause yield reduction. Plant height reduction was greater in the first rinse, followed by the second. No plant height difference was noticed between the third and the fourth rinse during the two evaluations. The first rinse suffered the major yield reduction, followed by the second and no difference was noticed between the third and follow up application. Data collected from this study suggest that for the dicamba formulations tested, triple rinse should be followed rigorously to avoid injury on non-DT soybeans.

List of Tables

Table 1. Year, location, varieties, and population class for each experiment

Year	Location	Variety	Population Seeds ha^{-1}
2020	Stapleton	Enlist Mycogen 209E	320,000
2021	North Platte	Syngenta S28-E3	320,000
2021	Lincoln	Syngenta S28-E3	320,000
2021	Tallassee	Pioneer p76t54r2	346,000

Table 2. P-values for main effects and interactions of rinse and mixture for HPLC data.

Fixed effects	Parts per million P-value
Rinse	<0.001
Mixture	0.006
Rinse*Mixture	0.0006

Table 3. Impact of number of rinses on HPLC data studies from a sprayer contaminated with different dicamba formulations.

Treatment	Rinse 1		Rinse 2		Rinse 3		Rinse 4	
	g ha^{-1}							
XtendiMax®	49.1	A	3.5	A	0.4	A	0.005	A
Diflexx®	33.6	B	3.5	A	0.4	A	0.002	A
Enginea®	25.5	B	2.1	A	0.4	A	0.008	A
Clarity®	33.0	B	2.3	A	0.4	A	0.003	A
Status®	16.0	C	1.3	A	0.4	A	0.04	A

Table 4. P-values for main effects and interactions of rinse and mixture for visual estimation of injury and yield reduction on soybeans during the evaluations across four replications in four studies.

	Visual estimation on injury				
	Days after treatment				Yield
Fixed effects	7	14	21	28	reduction
	P-value				
Rinse	<0.001	<0.001	<0.001	<0.001	<0.001
Mixture	0.490	0.320	0.051	0.065	0.341
Rinse*Mixture	0.130	0.845	0.140	0.397	0.175

Table 5. Impact of number of rinses on visual estimation of injury on soybeans at 7, 14, 21 and 28 days after treatment and yield reduction from a sprayer contaminated with different dicamba formulations across four replications in four studies.

	Visual estimation of injury[a]				
	Days after treatment				Yield
Rinse	7	14	21	28	reduction
	%				
1	60 A	64 A	70 A	75 A	83 A
2	29 B	35 B	36 B	39 B	14 B
3	13 C	16 C	16 C	20 C	1 C
4	0 D	0 D	0 D	0 D	-1 C

[a] Means within columns followed by a common letter are not significantly different according to Fisher’s Protected LSD test (α=0.05).

Table 6. P-values for main effects and interactions of rinse and mixture for plant height reduction on soybeans during the evaluations across four replications in four studies.

	Plant height reduction	
	Days after treatment	
Fixed effects	14	28
	P-value	
Rinse	<0.001	<0.001
Mixture	0.367	0.769
Rinse*Mixture	0.162	0.097

Table 7. Impact of number of rinses on plant height reduction on soybeans at 14 and 28 days after treatment from a sprayer contaminated with different dicamba formulations across four replications in four studies.

	Plant height reduction[a]			
	Days after treatment			
Rinse	14		28	
	%			
1	36	A	48	A
2	13	B	19	B
3	0	C	2	C
4	0	C	0	C

[a] Means within columns followed by a common letter are not significantly different according to Fisher's Protected LSD test (α=0.05).

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Chapter 4

Response of non-dicamba soybeans, cotton, tomatoes and peanuts to tank contaminations of dicamba tank mixtures of clethodim, and drift reducing adjuvants.

Abstract

The presence of herbicide resistance has become a challenge for row crop producers. To overcome this challenge, a new trait of dicamba-resistant soybeans and cotton broadened the spectrum of post emergent herbicides. With the increase in dicamba applications, thousands of reports of off-target movement have also occurred. Sprayer contamination has been identified as one cause of off-target movement of dicamba. Injury caused by superficial cleaning of sprayer tanks can vary from cosmetic to total yield loss and is directly related to growth stage, and amount of active ingredient present in the sprayer.

The objective of this study was to determine the effect of rinsates from different tank mixtures containing clethodim and dicamba with and without drift reducing adjuvants and in combination with a sprayer plumbing system cleaner on tomatoes, peanuts, cotton and non-DT soybeans in a greenhouse environment. Tanks were rinsed four times, with a sample collected after each rinse to simulate a triple rinse procedure and the follow up application. Rinse has shown to be the only significant effect in all evaluations during the study, neither interactions between solution and rinse. All the species responded with visual injury symptoms during the first three rinses but not in the 4th rinse using a full tank to simulate a subsequent application. Visual estimations of injury and dry weight reduction were higher in the first rinsate and followed by the second. Peanuts and cotton

recovered from the symptoms during the last evaluations. Soybeans and tomatoes have shown the most affected by the rinsates, being the first rinse the most problematic.

Introduction

Recent advances in biotechnology produced cultivars of cotton and soybeans resistant to dicamba, allowing the spraying of this herbicide over the top during the growing season (Behrens et al. 2007). The presence of glyphosate resistant weeds such as the pigweed family (*Amaranthus spp.)*, horseweed (*Erigeron canadensis L.*), and common ragweed (*Ambrosia atermisiifolia L.*), stimulated the incorporation of dicamba-resistant trait in the weed management program. (Wechsler et al. 2019).

Dicamba is a synthetic auxin part of the benzoic acid family. It is absorbed by roots and shoots and translocated in the phloem. Even though the translocation happens fast, plant death occurs 2 to 4 weeks after the exposure (Shaner et al. 2014). The classic symptomology of low rates of dicamba from off-target movement into sensitive crops are leaf cupping, stem, and leaf epinasty, cracked and swollen stems, chlorosis followed by necrosis (Kelley et al. 2005).

In a survey conducted by Werle et al. (2018), it was observed that not every farmer that planted dicamba-tolerant soybeans, sprayed this herbicide in their herbicide program. In these cases, this technology may have been adopted for protection against drift originated in neighbor's applications. Indeed, during 2017 growing season, 1.46 million hectares were reported with dicamba off-target movement (Bradley, 2017). In this year, four percent of all soybean fields in the United States suffered injury from off-target movement, Nebraska and Illinois being the largest share, with approximately 1 in every 13 fields suffering injury (Wechsler et al. 2019). Even though in 2018 this number was reduced to

445 thousand hectares (Bradley 2018) it is a recurring issue season after season.

The unintended damage can be caused mostly by particle drift, volatilization, and tank contamination (Matthews, 2014; Egan and Mortensen, 2012; Egan 2014). Particle drift occurs during application and decreases with downwind distance. However, it can be noticed causing a 1% visual injury estimation 293 meters from the edge of the field sprayed (Soltani et al. 2020). In contrast, volatilization can occur when gases are released from the sprayed area and settle in other sensitive fields (Egan and Mortensen 2012).

Sprayer contamination is caused by inadequate cleaning procedures. Even though dicamba is an herbicide that has high solubility in water (6.5 g L^{-1}), it can settle and dry in hard to reach parts of the sprayer leaving salt residues behind (Kamrin et al. 2010). Cundiff et al. (2017) analyzed different agricultural hoses and results show that dicamba retention varies among hoses and can have a significant role on soybean visual estimation of injury.

Marques et al. (2021) studied different tank materials (polyethylene and fiberglass) and concluded that dicamba was efficiently removed from both tanks if a triple rinse procedure was performed correctly. When spraying the rinsate on soybeans as a bio indicator, this study reported that plant height increases with each rinse, and yield reduction was noticed only in the first and second rinse. In addition, sprayer parts such as hoses can also become a source of contamination. Performing triple rinse with water and ammonia, Boerbom (2004) detected that 0.63% of initial concentration of dicamba was still present in the third rinse.

During the growing season, the same sprayer used to spray dicamba on tolerant crops, can be used to spray sensitive crops. The concept of having one sprayer specially dedicated to dicamba is the most efficient way to avoid sprayer contamination, but it is a costly solution. If tank cleanout is not followed , dicamba sub-lethal doses can be present in the sprayer and potentially cause damage and yield loss on susceptible plants.

Dicamba sub-lethal doses have been studied in a variety of crops such as snap bean, sweet potatoes, strawberry, watermelon, grapevines, pecan tree, apple, raspberry, peach trees and others (Colquhoun et al. 2017; Dintelmann et al. 2020; Culpepper et al. 2018; Shankle et al. 2021). However, there is no data available about how sensitive crops respond to rinsates from contaminated sprayers.

Thus, the objective of this study was to recognize the effect of tank-mixtures of dicamba and clethodim using different drift reducing adjuvants for sprayer rinsates from tanks cleaned with or without a detergent on soybeans, tomatoes, cotton, and peanuts. The hypothesis of this study was: (1) dicamba tank mixtures will affect the cleanout procedures; (2) triple rinsing the spray equipment will reduce dicamba concentration to an amount safe that will not cause injury in the follow up application in any of the species tested; (3) the use of tank plumbing cleaner will help reduce dicamba on the third and follow-up rinses.

Materials and methods

Spray application and tank clean out simulations and the greenhouse plant response studies were conducted at the Pesticide Application Technology Laboratory of the University of Nebraska-Lincoln in North Platte, Nebraska, USA in 2020 and 2021.

The system used to simulate tank cleanout consisted of two 189 L cone-bottom tanks equipped with Banjo polypropylene fitting (Banjo Corporation, Crawfordsville, IN) and a valve to ensure full drainage. Each tank mixture was recirculated through a diaphragm pump Shurflo® 2088-394-154 (Pentair, Minneapolis, MN) with an output of 12.1 L per minute and a 102 cm long synthetic rubber return hose was attached to an irrigation nozzle number 21 to equally distribute the water in the inner tank walls and ensure adequate residue removal.

Two tanks (tank 1 and tank 2) were first filled with 113 L of water, and tank mixture products next added in appropriate order. The solution circulated through the system for 20 minutes and disposed in a chemical tank reservoir. After the tanks were emptied, water was added at 10% of the initial tank volume and allowed to circulate through the system for 15 minutes before sample collection and solution disposal for each rinse. One sample of each rinsate was collected after the solution circulated through the system and a follow-up rinse was obtained by completing the tank to its initial volume (113 L of water) and allowing the solution to circulate for one minute before the collection. A sprayer plumbing system cleaner was added during the second rinse of appropriate treatments with the same collection parameters. Tank-mixtures producing rinsate from tank 1, provided solution to be sprayed over the first repetition of the study, and Tank-mixtures producing rinsate from tank 2 generated solution for the second repetition of the study.

Treatments included: XtendiMax® (Bayer CropScience LPP.O. Box 12014, 2 T.W. Alexander DriveResearch Triangle Park, NC 27709) at 1120 g ae ha^{-1} alone and in combination with either Intact® (5ml L^{-1}) (1429 S. Shields Drive Waukegan, IL 60085), Trap Line Pro II® (5ml L^{-1}) (CHS Inc. 5500 Cenex Drive Inver Grove Heights, MN 5507), or Select Max® (Valent USA Corporation, Walnut Creek, CA, 94596) at 136 g ae ha^{-1}. A triple tank-mixture of XtendiMax® + Select Max® and each drift reducing adjuvant was also incorporated. Each mixture was subject to a triple rinse cleanout procedure, either with or without the addition of WipeOut XS® (5 ml L^{-1}) during the second rinse. Samples were taken from each rinse, for a total of 52 treatments including four treatments with no products added to the solution for further comparison.

Five plants per treatment of tomatoes (*Lycopersicon esculentum*), soybeans (*Glycine max L. Merr*), peanuts (*Arachis hypogaea L.*) and cotton (*Gossypium hirsutum L*) were planted into cone pots filled with Pro-Mix BX5 (Premier Tech Horticulture Ltd, Rivière-du-Loup, Canada) general purpose growing medium, information about varieties can be found at Table 1. Plants were grown under a controlled greenhouse conditions with a daytime temperature ranging 26 to 28°C and a night temperature ranging 18 to 22°C.

Daylight period was extended to 16 hours using a LED light of 520 µmol s-1 (Philips Lighting, Somerset, NJ, USA). Plants were watered daily using a commercial liquid fertilizer (UNL 5-1-4; Wilbur-Ellis Agribusiness, Aurora, CO, USA) at 0.2% v v^{-1} incorporated with water.

Plants were sprayed when they were 20 to 25 cm in height using a single nozzle spray chamber calibrated to deliver 140 L ha^{-1} with an AI110015EV nozzle at 256 Kpal and 2.9 Km per hour. After application, plants were transferred to the greenhouse for visual injury evaluations and biomass weight. Visual estimations of injury were performed 7, 14, 21 and 28 days after application and the aboveground plant biomass done by harvesting the plants at 28 days after applications. Harvested material were kept in a drier at 65°C until constant weight was achieved. Dry biomass weight was converted into percentage of reduction and compared to the solutions that were sprayed with water using the following equation 1 (in which WT represent the mean biomass of the plants treated with water and the T represents the biomass of the treated plants):

$$\%\ Biomass\ reduction = \left[\frac{(WT - T)}{WT}\right] * 100$$

Data set was analyzed using PROC GLIMMIX in SAS 9.4 (Statistical Analysis Software, version 9.4, Cary, NC, USA) using solution and rinse as a fixed factor and study repetition as a random factor. All comparisons were performed using α = 0.05 significance using a Fisher's Protected LSD test.

Results and Discussion

Results indicate that dicamba concentrations are reduced each consecutive rinse performed. Including drift reducing adjuvants or clethodim to the tank did not shown any impact on dicamba removal from the tanks, the same trend was reported for the use of tank cleaners, resulting in no main effect significance for mixture in any parameters evaluated in any species.

Soybeans were significant effect for rinse ($P<0.05$) during all visual evaluations of injury and no mixture and interaction between mixture and rinse was noticed (Table 2). Visual estimation of injury decreases with each rinse. By seven DAT (Days after treatment) visual estimation of injury reported in the first rinse was 53%, in the second rinse 33%, third rinse 15 % and no injury was reported in any follow up evaluations. At 14 DAT, injury decreases statistically according to rinses, being 62%, 50% and 24% in the first, second and third rinse respectively. At the third week (21 DAT) the same pattern was observed, with 73%, 42 % and 23% visual estimations of injury for the first, second and third rinse respectively. At 28 DAT the visual estimation of injury for the first rinse was 73%, but it decreases to 53% and 32% in the second and third rinse, respectively (Table 3). Similar findings were reported by Browne (2020), where the use of triple rinse with water attain the same results using water and tank cleaner products.

Soybean dry weight reduction follows the same tendency as the visual ratings. The highest dry weight reduction was in the first rinse with 35% followed by the second rinse with 15% but no significant difference was noticed between the third rinse and follow up, resulting in 2% reduction and 0%, respectively. Similar findings were reported by Cundiff et al. (2019) where dicamba concentrations decreased with each rinse.

There was no significance interaction between tank mixture and rinse in cotton. Rinse was also the only significant effect for visual estimation of injury and dry weight reduction in cotton (Table 4).

Rinse visual estimations of injury and symptoms were diminished in each cleanout, not being present during any of the evaluation timings in the follow up application. At seven DAT injury were 32%, 13% and 6% for the first, second and third rinse, respectively. At 14 DAT the cotton plants presented the most injury from all evaluations, being 35%, 14.63% and 10% at the first, second and third rinse, respectively. At 21 DAT, visual estimations of injury were 33% for the first rinse, 12% in the second rinse and 6% at the third rinse. At 28 DAT plants demonstrated lower values of injury from the previous evaluation of visual estimation of injury with 31.88% at the first rinse, 9% at the second and 3% at the third rinse (Table 5).

The first rinse resulted in the most dry-weight reduction among all the rinses sprayed with 16.56% reduction. Second and third rinse were not significant different between them with 7.33% and 9.07% reduction respectively. Third and follow up rinses were not different. A field study conducted with different varieties of cotton has shown that sub-doses of dicamba and 2,4-D can cause visual injury and yield loss, however, dicamba has shown to be more sensitive to 2,4-D than to dicamba. Cotton showed visual estimation of injury but not yield loss in the lower doses, suggesting that low concentrations of dicamba might not affect yield, but tank contamination might be a source of off-target movement (Johnson et al. 2017).

Tomato visual injury response followed the same pattern observed for the other species with symptoms present in the first, second and third rinse but not in the follow up application (Table 6). The first rinse displaying the most injury, followed by the second and third rinse. At seven DAT visual estimation of injury were 56% for the first rinse, 16% for the second and 5% for the third rinse. At 14 DAT, the visual estimation of injury increased to 73%, 25% and 8% for the first, second and third rinse, respectively. At 21 DAT the third rinse had reduction in the symptoms with 4% of injury and the first and second rinse did not recover, being reported with 75% and 27% respectively. At 28 DAT, the first rinse increased its visual estimation of injury to 82%, second rinse reported 13% and the third rinse did not report any visual estimation of injury (Table 7).

The highest dry weight reduction impact was in the first rinse, with a total of 50.29% reduction, followed by the second rinse with 11.91% and no significant difference was reported between the third and follow up application with 0.09 % and -2.53% dry weight reduction.

Knezevic et al. (2018) spraying different doses of dicamba on tomatoes concluded that sub doses of dicamba can reduce the plant biomass by 50% and cause severe visual injury to the plants. In similar research, Kruger et al. (2012) describe that tomatoes are more sensitive to sub doses dicamba during the early vegetative stage (15 cm plant height) but exposure during early bloom stage resulted in greater yield losses.

The main effect rinse has shown significant during all visual evaluations on peanuts. No main effect significance for tank mixture was noticed either the interaction between the two main effects.

Peanut visual estimation of injury was higher in the first and second rinse, but still present in the third rinse (Table 8). Visual estimations of injury were higher at the first week and plants shown recovery until the last evaluation. No visual injury was reported at the follow up application. At seven DAT the visual estimation of injury was 17%, 8% and 4% for the first, second and third rinse respectively. At 14 DAT a recovery was noticed, reducing the visual estimation to 12% in the first rinse, 4% in the second rinse and 2% at the third rinse. At 21 DAT no injury was noticed in the third rinse and there was a reduction in injury in the first and second rinse to 8% and 2% respectively. At 28 DAT the only rinse that had symptoms was the first, with 6%, none of the other rinses shown symptoms during this evaluation (Table 9).

The only significant factor for dry weight reduction was rinse. The first rinse shown to reduce the dry weights by 8% while for the other rinses were not significant. Leon et al. (2014) reported that 35 g ae h^{-1} of dicamba is capable producing symptoms and reducing yield for peanuts. Johnson et al. (2012) sprayed low doses of dicamba on peanuts, soybeans, and cotton, reporting that doses necessary for causing symptoms and yield loss in soybeans are lower than in peanut and cotton.

Conclusion

Results from this study suggest that the combinations with drift reducing adjuvants and clethodim did not affect the dicamba removal from the tanks. Also, use of a tank plumbing system cleaner was not more effective than the results obtained with water. Visual estimation of injury reduces each subsequent rinse, and the highest dry weight reduction was observed in the first rinse. Results shown that peanuts recovered from the injury and did not show dry weight difference between the second, third and follow up. However, other species were more responsive when exposed to the rinsates, suggesting that if the sprayer is used for dicamba, a rigorous triple rinse procedure should be followed to guarantee that the other species will not suffer from the contaminated sprayer.

List of Tables

Table 1. Species and varieties used for the study

Crop	Variety
Soybeans	Asgrow 5253
Cotton	PHY 375 WRF
Tomato	Qualit 47 Kemterter products
Peanut	TUF Runner 297

Table 2. P-values for main effects and interactions of rinse and tank mixture for visual estimation of injury and dry weight reduction on soybeans during the evaluations across five replications in two studies.

	Visual estimation on injury				Dry weight reduction
	Days after treatment				
Fixed effects	7	14	21	28	
	P-value				
Rinse	<0.001	<0.001	<0.001	<0.001	<0.001
Mixture	0.930	0.108	0.800	0.386	0.086
Rinse*Mixture	0.999	0.124	0.927	0.527	0.268

Table 3. Impact of number of rinses on visual estimation of injury on soybeans at 7, 14, 21 and 28 days after treatment and dry weight reduction at 28 days after treatment from a sprayer contaminated with different dicamba tank mixtures across five replications in two studies.

Rinse	Visual estimation of injury[a]				Dry weight reduction
	Days after treatment				
	7	14	21	28	
	——————— % ———————				
1	52 A	62 A	72 A	72 A	35 A
2	32 B	50 B	42 B	52 B	15 B
3	15 C	24 C	32 C	32 C	2 C
4	0 D	0 D	0 D	0 D	0 C

[a] Means within columns followed by a common letter are not significantly different according to Fisher's Protected LSD test (α=0.05).

Table 4. P-values for main effects and interactions of rinse and tank mixture for visual estimation of injury and dry weight reduction on cotton during the evaluations across five replications in two studies.

	Visual estimation on injury				
	Days after treatment				Dry weight reduction
Fixed effects	7	14	21	28	
	P-value				
Rinse	<0.001	<0.001	<0.001	<0.001	<0.001
Mixture	0.183	0.050	0.183	0.497	0.313
Rinse*Mixture	0.969	0.968	0.999	0.250	0.477

Table 5. Impact of number of rinses on visual estimation of injury on cotton at 7, 14, 21 and 28 days after treatment and dry weight reduction at 28 days after treatment from a sprayer contaminated with different dicamba tank mixtures across five replications in two studies.

Rinse	Visual estimation of injury[a]								Dry weight reduction	
	Days after treatment									
	7		14		21		28			
	%									
1	32	A	35	A	33	A	32	A	16.56	A
2	13	B	14	B	12	B	9	B	7.33	BC
3	6	C	10	C	6	C	3	C	9.07	B
4	0	D	0	D	0	D	0	D	2.59	C

[a] Means within columns followed by a common letter are not significantly different according to Fisher's Protected LSD test (α=0.05).

Table 6. P-values for main effects and interactions of rinse and tank mixture for visual estimation of injury and dry weight reduction on tomatoes during the evaluations across five replications in two studies.

	Visual estimation on injury				
	Days after treatment				Dry weight reduction
Fixed effects	7	14	21	28	
	P-value				
Rinse	<0.001	<0.001	<0.001	<0.001	<0.0001
Mixture	0.9467	0.7447	0.3414	0.5912	0.1128
Rinse*Mixture	0.9503	0.1719	0.8803	0.4438	0.1244

Table 7. Impact of number of rinses on visual estimation of injury on tomatoes at 7, 14, 21 and 28 days after treatment and dry weight reduction at 28 days after treatment from a sprayer contaminated with different dicamba tank mixtures across five replications in two studies.

	Visual estimation of injury[a]				
	Days after treatment				Dry weight
Rinse	7	14	21	28	reduction
	%				
1	56 A	73 A	75 A	82 A	50 A
2	16 B	2 B	27 B	13 B	12 B
3	5 C	8 C	4 C	0 C	0 C
4	0 D	0 D	0 D	0 C	-3 C

[a] Means within columns followed by a common letter are not significantly different according to Fisher's Protected LSD test (α=0.05).

Table 8. P-values for main effects and interactions of rinse and tank mixture for visual estimation of injury and dry weight reduction on peanuts during the evaluations across five replications in two studies.

	Visual estimation on injury				Dry weight reduction
	Days after treatment				
Fixed effects	7	14	21	28	
	P-value				
Rinse	<0.001	<0.001	<0.001	<0.001	<0.001
Mixture	0.998	0.748	0.341	0.591	0.055
Rinse*Mixture	0.999	0.172	0.880	0.444	0.845

Table 9. Impact of number of rinses on visual estimation of injury on peanuts at 7, 14, 21 and 28 days after treatment and dry weight reduction at 28 days after treatment from a sprayer contaminated with different dicamba tank mixtures across five replications in two studies.

	Visual estimation of injury[a]				
	Days after treatment				Dry weight reduction
Rinse	7	14	21	28	
		—— % ——			
1	17 A	73 A	75 A	82 A	8 A
2	8 B	25 B	27 B	12 B	-1 B
3	4 C	8 C	4 C	0 C	-2 B
4	0 D	0 D	0 D	0 C	0 B

[a] Means within columns followed by a common letter are not significantly different according to Fisher's Protected LSD test (α=0.05).

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