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EFFECTS OF TANK CONTAMINATION AND IMPACT OF DRIFT-REDUCING AGENTS ON WEED CONTROL IN RESPONSE TO DICAMBA APPLICATIONS

by

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EFFECTS OF TANK CONTAMINATION AND IMPACT OF DRIFT-REDUCING AGENTS ON WEED CONTROL IN RESPONSE TO DICAMBA APPLICATIONS

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Availability of dicamba-tolerant (DT) crops from 2017 provided farmers with additional herbicides for weed control management in row crops. However, the technology alike this one has concerns regarding dicamba off-target movement (OTM) causing undesirable effects on sensitive vegetation. Even though dicamba has high water solubility OTM that has often been overlooked when it comes to unintended crop exposure is dicamba tank contamination. Considering the complexity of spraying equipment soybean response may be expected even when small amounts of residues are left in the spray equipment. Typically, the same field spray equipment is used to perform herbicide application through growing season there is a limited knowledge how various postemergence (POST) programs impacts soybean response when found in scenario with dicamba tank contamination and requires additional research.

Furthermore, as one way to mitigate OTM potential release of DT crops was followed with registration of various agents also known as drift-reducing agents (DRAs). Increased awareness of both growers and commercial applicators to reduce unintended adjacent crops injury use of labeled DRAs in combination with drift-reduction nozzles represent common practice. Exposure of sensitive crops to sublethal doses of dicamba has been well documented over several years; however, there is limited information available how combination with commonly used DRA's may impact application process and weed control. Considering limitations on available literature the main objective of this research were: 1) evaluate response of non-DT soybean variety when exposed to commonly applied POST herbicide program in combination without or with dicamba as tank-contaminant and 2) evaluate impact of DRAs on weed control in response to dicamba applications. The results of this research expanded knowledge and will help in education in the future management decisions about potential implications associated with common mitigation techniques used with dicamba application as well as helped with understanding how various POST herbicide program affect soybean response.

Key words: RR-soybean, EPSPS, PPO, ACCase, Sprayer cleanout, Synthetic auxins, DRAs, Off-target movement, Drift, Efficacy

Dedication

For my family, siblings, and everyone who believed in me.

"Farming looks mighty easy when your plow is a pencil, and you're a thousand miles from the corn field."

- *Dwight D. Eisenhower*

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CHAPTER 1

Literature Review

Soybean (*Glycine max* (L.) Merr) represents one of most important agricultural crops in terms of global value. Originally from China, soybean was first reported in America during early 1900s (Dies 1942). The majority production of soybean has historically been greatest in the United States, Brazil, and Argentina. According to the United States Department of Agriculture (USDA) those countries account for nearly 80 percent of world soybean production (USDA ERS 2016). Increased planting flexibility and steadily rising yield favored expansion of soybean acreage in the United States. However one of the most limiting factors for soybean production are considered weeds. Oerke (2006) estimated that about 37% of achievable soybean production is reduced by weed competition, compared to 11, 11, and 1% by pathogens, animal pests, and viruses, respectively. Direct competition for light, water, nutrients, and space in row crops can drastically reduce soybean quality and quantity. Development of a well-organized and conducted weed control programs is a key for successful weed management and sustainable production.

As the second-most-planted crop in the United States, about 94% of the total area planted with soybean are herbicide-tolerant cultivars (USDA ERS 2019). Commercialization of glyphosate-tolerant soybeans in 1996 completely changed previous weed management practices and signaled the beginning of a new era of weed management in row crops (Dill 2005). The adoption of soybean herbicide-tolerant varieties (Roundup Ready®) has resulted in a shift of practices toward greater reliance on postemergence (POST) herbicide program, and more specifically, glyphosate use. Changing management practices in this way significantly influenced the composition of weed species communities followed with higher selection pressure and evolution of resistance weed (Heap 2014, Vencill et al. 2012).

Unfortunately, overreliance on glyphosate for POST weed control has led to the development of glyphosate-resistant (GR) weed biotypes (Owen 2008). Currently, in the United States 165 resistant weeds have been reported of which 17 are reported as GR (Heap 2019). Without effective POST herbicide options, there is a high potential for yield loss due to interplant competition (Terra et al. 2007). Therefore, alternative herbicide management options that will decrease selection pressure and slow down the evolution of weed resistance are necessary.

Approval for use of dicamba over the top of dicamba-tolerant (DT) crops represents a tremendous change in the field of agriculture. Plant growth regulators such as dicamba belongs to the group 4 site of action group which refers to synthetic auxin herbicides (WSSA 2014). Prior to 2016, application of herbicide products containing dicamba was limited in use as either a burndown or POST treatment in corn, sorghum, small grains, pasture, rangeland, and turf grass. After 2016, dicamba and other similar herbicides from this group can now also be applied over the top in soybean and cotton. Having possibility to be applied in broadleaf crops it was expected to have increased of dicamba use over the past several years (USGS 2020).

The introduction of crops tolerant to synthetic auxins was primarily initiated due to excellent plasticity since their development. After over five decades of use, 17 dicamba-resistant weeds have been documented (Heap 2020). Commercialization of DT crops enabled growers to apply this chemical POST with more flexible application timings and potential to control weed species that germinate later in the season (Werle et al. 2014). Having the possibility to be applied later with the possibility for residual weed control associated with some products available on the market, this herbicide provides growers with a site-of-action that is highly effective for some of the troublesome herbicide-resistant broadleaf weeds.

Examples of troublesome broadleaf controlled by dicamba includes giant ragweed (Ambrosia trifida L.), common ragweed (*Ambrosia artemisiifolia* L.), common lambsquarters (*Chenopodium album* L.), pigweed species (*Amaranthus spp.*), velvetleaf (*Abutilon theophrasti* Medik.), common cocklebur (*Xanthium strumarium* L.), and horseweed (*Conyza canadensis* (L.) Cronq.). Each has been reported as resistant to one or more herbicide site-of-actions (Heap 2014, Jhala et al. 2014, Kniss 2018a, Mithila et al. 2011, Vieira et al. 2018). In order to mitigate present problem with herbicide-resistant biotypes, chemical companies recently developed and released genetically modified crops tolerant to dicamba (Taylor et al. 2017). Currently, four herbicide products are labeled for use in dicamba-tolerant crops: Xtendimax® with VaporGrip Technology® (Bayer Crop Science), Engenia® (BASF), FeXapan® plus VaporGrip Technology® (Corteva Agroscience), and Tavium® plus VaporGrip Technology® (Syngenta).

According to the USDA-NASS (2018), an estimated 2.3 million hectares of soybeans were planted in Nebraska in 2017. Werle et al. (2018) reported about 19% of those acres were planted with DT soybean varieties. Respondents to Werle's survey anticipated acres of DT soybean would increase up to 52% of total acres planted in 2018. This was later confirmed by the USDA (2018) having DT varieties planted on about 1.2 and 1.6 million ha^{-1} during 2018 and 2019, respectively. A potential reason for the rapid adoption of DT soybean may be due to the significant reduction of possible crop injury and potential yield loss from adjacent fields planted with sensitive varieties (Hurley and Frisvold 2016).

Considering that this system is getting widely adopted, it is likely that risk for unintended crop exposure will increase. Sensitive broadleaf plants include non-dicamba-tolerant (non-DT) soybean (*Glycine max* (L.) Merr.) (Weidenhamer et al. 1989), and cotton (*Gossypium hirsutum* L.) (Marple et al. 2008), sunflower (*Helianthus annuus* L.) (Derksen 1989), peanut (*Arachis hypogaea* L.) (Johnson et al. 2012), wine grape (*Vitis vinifera* L.) (Al-Khatib et al. 1993), tomato (*Lycopersicon esculentum* L.) (Kruger et al. 2012) and many other crops, orchards and ornamental plants. Primary ways how unintended injury may occur are through physical particle drift (Alves et al. 2017) , secondary off-target movement through either droplet suspended in the air or volatility (Bish et al. 2019) and tank contamination (Soltani et al. 2016).

Particle drift represents the part of a pesticide application that moves away from the target area by site specific wind velocity and direction (Ebert et al. 1999, Matthews et al. 2014). Generally, soybean is among the most susceptible crops to growth regulators. Soybean response to doses which are multiple thousand times less than recommended label dose has a potential to affect all sensitive parts causing malformations such as changes on leaf parenchyma, leaf cupping or steam twisting (Auch and Arnold 1979; WSSA 2014). Furthermore, increasing synthetic auxin concentration in the plant tissue combined with inability of non-DT varieties to metabolize those synthetic auxins causes abnormal plant growth and ultimately plant death (Hansen and Grossmann 2000). The primary disadvantage of sensitive crop exposure to dicamba is it may lead to significant economic losses (Andersen et al. 2004, Kelley et al. 2005). Damage caused by dicamba is can be attributed due to abnormal growth and cell division typical of synthetic auxins. Having uncontrolled growth eventually triggers the collapse of the vascular tissue followed by the plant death (Kelley and Riechers 2007). The level of crop response as mentioned is likely to be dependent on the amount of dicamba that reached the soybean and the growth stage of plant when exposure occurred (Solomon and Bradley 2014).

Another potential source of dicamba secondary off-target movement is volatility. Currently available dicamba products include formulations capable to volatilize having for some of them more than for others (Bish et al. 2019). When applied under high temperature followed by low relative humidity, secondary movement or vapors released from this chemistry can easily move from treated areas onto susceptible advancement fields and cause crop injury (Behrens and Lueschen 1979, Egan and Mortensen 2012). Mitigation of problem with crop injury chemical companies developed two formulations with a goal to minimize volatility potential. New formulations includes *N*,*N*-bis-(3-aminopropyl)methylamine salt which is also known as BAPMA salt and diglycolamine (DGA) salt with a VaporGrip technology that contains acetic acid buffer and helps with formulation stability when exposed to various environmental conditions (Abraham 2018). Even though, introduction of new formulations significantly decreased potential for volatilization, this phenomenon has not been eliminated and may cause response to soybean and other prone to injury plants (Bish et al. 2019).

One method of off-target movement that has often been overlooked when it comes to unintended crop injury is dicamba tank contamination. Similarly, with all other previously mentioned ways for soybean exposure, dicamba tank contamination can also result in symptomology with doses thousands of times lower than the standard utilized dose. The main issue for this may be due to the possibility that applicators typically use the same field spray equipment to perform application of different pesticides for pest management. Following this, injury can occur when even small amounts of residues are left in the spray equipment. This can happen when an incomplete tank cleanout after treated either resistant or tolerant crops.

Additionally, consider the complexity of spraying equipment which uses various materials and connections among sprayer tank and boom for solution discharge. Having diversity among all hoses and plumbing connections built into the spray system may result in different porosity of materials where just using water alone for spray cleanout may not be efficient (Johnson et al. 1997). Available research shows that significant difference in cleanout procedures may be observed among various hose types due to different porosity of material used for manufacturing (Cundiff et al. 2017). Even though, according to Kamrin (2010), dicamba has high water solubility (6.5 g L^{-1}), these products may settle and dry to different hard to reach areas of sprayer system leaving salt residues, which ultimately makes cleaning procedures more difficult. Following a standard procedure for sprayer clean out having triple rinse with water after applying this chemistry may represent scenario with high risk for off-target movement leaving high enough concentration of dicamba residues in the system to cause response on susceptible plants like soybeans (Osborne et al. 2015) .

Soybean exposure timing may be considered as an important factor when it comes to susceptibility to dicamba. With a flexibility in application, late POST applications of dicamba are more predisposed to result in unintentional sensitive crop injury if compared to burndown and early POST applications. A meta-analysis conducted by (Egan et al. 2014) showed that visual symptoms on soybean caused with dicamba drift during vegetative stage were not considered as indicator of final yield loss. Additional findings show that susceptibility of soybean to dicamba increases at the flowering stage or later in the season (Wax et al. 1969; Auch and Arnold 1978; Egan et al. 2014; Kniss 2018, Soltani et al. 2016).

Finally certain tank-mixtures as well may result in changes of solution pH and shifting ratio towards droplets that are more prone to drift can occur (Meyer et al. 2016, Mueller and Steckel 2019). One method to mitigate off-target movement of dicamba was the development and registration of various drift reducing agents (DRA) for use with dicamba applications, drift reducing nozzle types, and limitations on application parameters (operated pressure and boom height) suggested by product labels. Increased awareness of both growers and commercial applicators to reduce environmental contamination and non-DT sensitive crop exposure typically requires employment use of labeled DRA's in combination with drift reduction nozzles. Even though, these agents are used primarily for drift mitigation, their function when found in tank-mix has been often includes other functions like water conditioning, surfactants, defoamers, humectants, pH modifiers, etc. The addition of any material into tank-mixture may modify significantly spray characteristics (Oliveira et al. 2015, Prokop and KEJKLÍČEK 2002, Spanoghe et al. 2007). Dorr et al. (2013) reported changes in properties of spray mixture in combination with nozzle type and various operating parameters have significant impact on any pesticide application. Relationship of how changes in physicochemical properties may influence application process with the addition of commonly applied DRA requires additional attention to understand better the impact on biological efficacy of some of the troublesome weed species found in row crops.

Purpose of Research

Although dicamba tank contamination was initially believed to not be a primary cause for unintended injury to dicamba-sensitive soybean, with the continuous increase in cropping area planted with dicamba-tolerant soybean over the last several years and the consequent increase in dicamba use, it seems clear that tank contamination plays a major role the issues related to dicamba off-target movement. Having limitations on knowledge how various POST programs impacts soybean response when found in scenario like dicamba tank contamination requires additional research.

Further, the release of DT crops and new dicamba formulations of dicamba were followed by registration of various adjuvants targeting the mitigation of some of the issues associated with off-target movement. The addition of DRAs with certain tank-mixtures that include dicamba has been required by law. However, the impact of these chemistries on the application process when using drift reducing nozzles is not well understood.

The objectives of this research was to evaluate through field and greenhouse experiments: (1) Effects of dicamba simulated tank contamination with various commonly used POST herbicide programs applied over the top of non-dicamba-tolerant soybean; and (2) the biological response of velvetleaf (*Abutilon theophrasti* Medicus) and common lambsquarters (*Chenopodium album* L.) with approved nozzle types for dicamba application with commonly applied DRA’s to identify the potential interaction on spray pattern associated with different operating parameters.

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CHAPTER 2

Dicamba Simulated Tank-contamination in Common Postemergence Herbicide Applications on Non-dicamba-tolerant Soybean

Abstract

Development of dicamba-tolerant (DT) crops was driven by a need for viable herbicide options for postemergence (POST) weed control in soybean. Even DT crops provided farmers with a feasible approach to control troublesome weeds there are several concerns related with off-target movement. Growers have believed that dicamba tank contamination was not a primary cause for to non-dicamba-tolerant soybean exposure. However, with the continuous increase in planted area with DT soybean it seems clear that tank contamination may play a major role related to dicamba off-target movement (OTM). The main objective of the field experiments conducted during 2018 and 2019 was to determine the impact of commonly applied POST herbicides with simulated multiple levels of dicamba as tank contaminant (0, 0.056 and 0.560 g ae ha^{-1}) during vegetative and reproductive stage on soybeans. Result from this study show most POST herbicides applied were detrimental for visible symptomology. Intensification of symptoms was observed exponentially as dicamba dose was increased. Comparing all site of action herbicides tested PPO-inhibiting herbicides had greater impact on soybean visual response, plant height reduction and final yield. Determining the impact of dicamba contamination when using various herbicide programs for weed management highlights the importance of proper sprayer cleanout following dicamba applications.

Introduction

Difficulties to create new active ingredients with unique modes of actions has resulted in development of genetically engineered crops tolerant to herbicides. Continuous use of herbicides with the same mechanisms of action over the time has led development of herbicide-resistant weeds. Currently in worldwide has been reported 573 resistant weed species (Heap 2019). With the limitations on currently available options for POST weed control additional tools were needed to decrease selective pressure and evolution of weed resistance.

As a one way to integrate more diverse chemical options for management of troublesome weeds companies released herbicide-tolerant crops to dicamba (Taylor et al. 2017). Historically by 2016 application of products that contained dicamba was allowed just in pastures, corn, small grain crops, sorghum, landscape, and rangeland maintenance (WSSA 2014). However, after development and release of soybean and cotton with trait to tolerate dicamba allowed application over the top in row crops. According to the Federal laws dicamba application in soybean has been currently allowed to be applied either 45 days after planting or at the beginning of bloom (R1) whatever comes first with some states being more restrictive with cut-off application dates than others (Anonymous 2018).

Having flexibility in application followed by excellent weed control potential for the adoption of soybean technology was expected to be significantly adopted by soybean growers nationally. Just in Nebraska planted area under DT varieties represented about 39% and 52% of the entire area planted with soybeans or about 1.2 and 1.6 million ha^{-1}, for 2018 and 2019 growing season, respectively (USDA NASS 2018, USDA - ERS 2019). As a potential reason for broad adoption might be due to wide presence of species that has been reported as resistant to EPSP Synthase Inhibitors (Vieira et al. 2018), PPO inhibitors (Vieira et al. 2017), ALS inhibitors (Heap 2020), HPPD inhibitors (Jhala et al. 2014), and others.

However, from the 39% planted with DT soybean in Nebraska during 2018 growing season just 27% of it have been sprayed over the top (USDA - ERS 2019). Release of row crop like soybean tolerant to dicamba provided growers with an alternative herbicide management options for weed control. However, there are several OTM concerns associated with use of this chemistry. Tank contamination, physical particle drift, and volatility are among several factors that may contribute to OTM and cause potential unwanted effects on sensitive vegetation (Alves et al. 2017, Behrens and Lueschen 1979, Bish et al. 2019, Soltani et al. 2016, Strachan et al. 2013). Potential reasons for not performing application over the top for the entire planted area with tolerant crop may be due to reduction of probability for soybean or other sensitive crop exposure planted in adjacent fields (Hurley and Frisvold 2016).

In order to sustain high level of production various pesticides has been applied using an existing equipment (Werle et al. 2018). Regardless scenario who performs pesticide application typically the same spray equipment has been used to perform various pest management treatments (i.e. herbicides, insecticides, fungicides. etc.). Without proper sprayer clean out after dicamba application residues left in hard to reach parts of the sprayer (tank, hoses, screens, boom, nozzles with carousels, etc.) or external tanks and shuttles used for mixing and delivering tank-mixtures represents doses that are about thousand times less than standard utilized doses.

Available survey conducted during 2018 Nebraska’s soybean growers consider dicamba tank contamination as one of the least important whereas OTM such as volatilization and physical particle drift were considered as the main cause non-DT soybean and other sensitive plant exposure (Werle et al. 2018). Although, tank contamination has not been considered as primary way for unintended soybean exposure with continuous increase of soybean area planted with DT varieties over the last several years and increased dicamba use it is inevitable that tank contamination will become an issue.

Dicamba is a highly water soluble (6.5 g L^{-1}) active ingredient; however, with various materials that are commonly used to build complexed mixing/spraying systems use of just water for clean out may not be adequate if followed usual recommendation that involves triple rinsing if considered used materials typically are with highly divergent porosities (Johnson et al.1997, Karmin 2010). Cleanout procedures using just water with the addition of ammonia to clean out sprayer system the amount of dicamba that has been recovered from spray boom represented 0.63% of initial spray mix (Boeroom 2004). Studies conducted by Cundiff et al. (2017) shows that dicamba persistence using various hose types may play significant role in visual response when using a soybean as indicator plant. Having various porosity among materials after dicamba use and having residues that may not be cleaned thoroughly in some of the sprayer hard to reach areas making cleaning procedures considerably more difficult. According to Golus (personal communication) dose of 1/250.000 of the standard utilized dose of 560 g ae ha^{-1} under controlled conditions resulted in a visible soybeans symptoms.

Symptomology of soybean plants when exposed to synthetic auxin herbicides has been reported rather as dose dependent (Auch and Aronold 1978, Kelley et al. 2005). Some of the symptoms when exposed thousand times less of the recommended labeled dose of 560 g ae ha^{-1} includes leaf cupping of terminal leaves and changes on leaf parenchyma with possibility that growth may be affected (Andersen et al. 2004, Behrens and Lueschen 1979). Even though, that soybean symptoms may be severe if apical meristem was not affected symptomology cannot be considered as prediction for final yield especially if symptoms were observed early in the growing season (Egan et al. 2014, Kniss 2018, Robinson et al. 2013). Estimations have been made that dicamba dose that cause about 30% visible injury to soybean early in the growing season appear implausible that yield will be affected (Kniss 2018).

For example, available studies show that when dicamba was applied at 5.8 g ae ha^{-1} at V2 growth stage resulted in response greater than 40% was followed with soybean yield loss of 5% (Soltani et al. 2016). With more flexible application timing and soybean capability to withstand exposure to dicamba early in the season in comparison with the beginning of flowering stage (R) sensitivity increases for about two to six-fold (Egan et al. 2014, Griffin et al. 2013, Kelley et al. 2005, Kniss 2018). When exposed to dicamba dose of about 0.9 g ae ha^{-1} there is a potential to result in significant visual response. Visual estimation of symptoms at R growth stage reported as greater than 12% was estimated to result at least in 5% of the yield loss (Kniss 2018). Studies which included exposure of glyphosate-tolerant soybean during reproductive stage with dicamba at 1 g ae ha^{-1} resulted in 23 – 28% in visual response with 5% of soybean yield loss (Soltani et al. 2016). With increased sensitivity later in the growing season dicamba doses significant changes on the plants or complete death of the growing points were reported as significantly lower than ones when soybean plants were exposed during early vegetative stages (Kniss 2018, Robinson et al. 2013, Soltani et al. 2016). Additionally, dicamba exposure at R stage plant height estimations may be considered as quick estimate for the yield loss (Weidenhamer et al. 1989). Having significant reduction of plant height greater yield loss may be expected due to significantly reduced formation of reproductive structures like number of nodes, flowers, pods and seeds per plant has been often reported as highly correlated with soybean yield (Robinson et al. 2013).

Currently, there are few available studies that investigated the impact of dicamba presence as tank contaminant; however, there are even less studies that reported impact of dicamba in non-DT crops when found in different tank-mixtures which growers often use to control pests. Determining the impact of simulated contamination on non-DT cultivar with various POST herbicide program that farmers might use in soybean will help in education about the importance of proper sprayer cleanout and its impacts on future management decisions.

Hypothesis of this study were: (1) soybean response to simulated dicamba tank contamination exposure will be influenced by growth stage; (2) soybean response will be influenced by various sublethal dicamba doses used for tank contamination; and (3) commonly applied herbicides will have different response when applied in a combination with sublethal dicamba doses.

Material and Methods

Two field studies were conducted during summer 2018 and 2019 growing season at the West Central Research and Extension Center in North Platte, Nebraska (41° 05’17.2” N - 100° 46’40.7” W). Soil type at this site was Sandy Loam with a sand, silt, and clay percentage of 57, 32, and 11 %, respectively, and a pH of 7.5.

Soybeans were grown in no-till system in crop rotation after corn. Hoegemeyer variety of soybean (2511NRR) was planted on May 24, 2018 and May 15, 2019 with a planting rate of 345.000 seeds ha^{-1}. All plots including non-treated control kept weed free throughout the season using a combination of herbicides and cultivation. All maintenance herbicides were applied using a four-wheeler equipped with flat-fan nozzles spaced 50 cm apart calibrated to delivered 187 L ha^{-1} at 276 kPa. After soybean rows closed hand weeding was performed as required.

In order to prevent cross contamination between plots entire area with soybeans planted (about 1.3 ha^{-1}) was divided into two subsections to evaluate soybean exposure during vegetative and reproductive growth stage. Experimental units (plots) were consisted of six 76 cm wide planted soybean rows (Figure 1) and 7.6 m (2018) and 10 m (2019) long. Field trials were established in a randomized complete block design that consisted of four replications (following the pH gradient in the field) with factorial arrangement of treatments (Table 1). Two treatment factors included 13 POST herbicides and three simulated level of dicamba as tank contaminant. Evaluated treatments included non-treated control, two 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) inhibitors, five Acetyl CoA Carboxylase (ACCase) inhibitors, three protoporphyrinogen oxidase (PPO) inhibitors, and crop oil concentrate (COC). Each treatment had three simulated doses of dicamba as tank contaminants (0, 0.056, and 0.560 of the label dose 560 g ae ha^{-1}). Treatments were applied separately in the assigned fields when soybean developed third trifoliate (V3), and second when soybeans were at the beginning stage of flowering (R1). Application was performed using a CO_2 backpack sprayer equipped with a six-nozzle boom equipped with Air Induction Extended Range (AIXR) 110015 nozzles calibrated to deliver 140 L ha^{-1} of a solution at 345 kPa. More details about application conditions through duration of application may be found in Table 2 and environmental conditions throughout the 2018 and 2019 growing seasons in Table 3.

After application response variable that were recorder included soybean symptomology 21 days after application (DAA), plant height 21 DAA and soybean height at the harvest time. Even though, Behrens and Lueschen (1979) established a scale for visual evaluation after soybean were exposed to dicamba the lack of use the same scale for this study may be attributed to presence of various POST herbicides had an impact on final soybean response. Further, soybean symptomology was estimated based on whole plot response using a scale 0 – 100%, where 0% represented no visual response and 100% was complete crop death. For soybean plant height of four completely random plants (rows two and five) were measured from the soil surface to the top of the main stem of the plant. When soybean plants developed all pods and plant senescence started a sample of six completely random plants were taken to record number of pods per plant using plot rows two and five. Soybean plot rows three and four were harvested using a two-row research plot combine and yields were adjusted to 13% of moisture. Additionally, individual samples from harvester were collected in order to determine if there is an impact of applied treatments on 100-seed weight which further in text referred as grain weight.

Data Analysis. Within each soybean growth stage, data across growing seasons was subjected to joint analysis using Sisvar Statistical Software, version 5.6 (Ferreira, 2011). For each variable, a comparison was made between the Root Square Mean Error (RSME) from the two seasons (data not shown). As the ratio between the highest and lowest RSME was smaller than 3 (Box, 1954) for all variables, the data points were combined across growing seasons.

Normality of residuals and homogeneity of variance of data (Table S1) were analyzed using the Kolmogorov-Smirnov and Levene’s tests, respectively, using SPSS Statistical Software, version 20 (SPSS Inc., Chicago, IL, USA). Based on this assumption analysis, transformation of data was not necessary. Data was subjected to analysis of variance (ANOVA) using Sisvar Statistical Software (version 5.6). Postemergence herbicides and simulated levels of dicamba doses were compared to each other using the Scott Knott and Tukey’s multiple comparison tests, respectively. A Scheffé’s contrast test was performed on yield data using the Sisvar Statistical Software, version 5.6. Additionally, response variables were correlated to each other by Pearson’s correlation test using the SPSS Statistical Software, version 20. All comparison tests were performed at $\alpha = 0.05$ significance.

Results and Discussion

Soybean exposure at vegetative (V3) growth stage. Analysis of variance (Table 4) for all evaluated parameters for soybean exposed to various POST herbicide tank-mixtures with simulated sublethal dicamba doses had a significant interaction between herbicide and dicamba dose ($P<0.0001$) for soybean symptomology, plant height at 21 DAA, harvest plant height, and yield. Further, yield components such as number of pods and grain weight (100 seed weight) were not affected by either herbicide applied or simulated dicamba dose used. Observed two-way (herbicide*dicamba dose) interaction will be discussed in both ways regardless either of herbicide applied within each dicamba dose and across all dicamba doses for herbicides applied over the top of soybean.

Soybean symptomology at 21DAA. Symptomology observed for soybean plants exposed to dicamba during V3 growth stage included leaf crinkling and cupping of terminal leaves as a result of exposure to low doses of dicamba (Foster and Griffin 2019) followed with foliar necrosis for used contact herbicides. Based on visual estimation of soybean symptomology at 21 DAA for POST herbicides mixed with sublethal doses of dicamba shows presence of dicamba in tank-mixture may intensify plant response.

Results presented in Table 5 indicates visible symptomology exponentially increases as dicamba dose increased. After treatment application over the top of the soybean response was no different when there was no dicamba present in tank-mixture and when found at 0.056 g ae ha^{-1} for EPSP synthase, ACCase inhibitor herbicide tested, and COC. Similar findings from available literature associated with treatment that dicamba alone at 0.06 g ae ha^{-1} resulted in less than 5% in visible response (Robinson et al. 2013). According to (Kniss 2018) estimated dicamba dose that cause about 5% of visual symptoms was determined to be about 0.038 g ae ha^{-1}. Considering broad diversity among soybean response reported in literature associated with the same or slightly different dicamba levels due uncertainty associated with visual appearance of symptoms and having some of them invisible for human eyes other most reliable methods like use of hyperspectral cameras were suggested (Zhang et al. 2019). Having diversity in soybean response among studies previously mentioned it may be concluded that soybean response to dicamba may be extremely challenging to evaluate. Regarding the data obtained in present study one of the main conflicting results that can be found in literature regarding to soybean visual response for soybean exposure to dicamba at V3 growth stage for both dicamba alone (0.056 g ae ha^{-1}) and with the addition of 1% v v^{-1} C.O.C where authors reported about 90% of visual symptoms (Andersen et al. 2004). With limited information how treatments were applied these findings partially may be explained with dicamba formulation used for treatment application as well as variety planted and evaluated for each individual study.

Additional findings from this study shows difference in plant response was found when dicamba was present at 0.560 g ae ha^{-1}. Dicamba alone at this dose in resulted in 9% of visible soybean response. Literature finding associated with doses of dicamba slightly higher if compared with one that have been used in this study 0.750 g ae ha^{-1} resulted in soybean visual response approximately about 20% at 28 days after application (Soltani et al. 2016). The addition of various herbicides in mixture significantly increased plant response with differentiation in plants response when various POST products for weed control were added in tank-mixture. Active like clethodim formulations where applied at of 280 g ai ha^{-1} resulted in soybean symptoms greater than 30% which if compared with other applied treatments was the lowest.

As expected, herbicides from site of action group 14 resulted in considerably higher soybean response even with base treatment that did not contained dicamba. When exposing soybean to product like acifluorfen observed symptoms were significantly different across dicamba doses and resulted in visible response about 11, 16, and 37% for dicamba doses 0, 0.056, and 0.560 g ae ha^{-1}, respectively. Similar findings associated with intensification of soybean response were reported by Kelley et al. (2005) when using 330 g ai ha^{-1} with an addition of 5.6 g ae ha $^{-1}$ of dicamba where soybeans visual estimation of injury was significantly different than both non-treated control and POST herbicide treatment that included just application of fomesafen alone over the top of the soybeans. Nevertheless, these findings has been contradictory from available literature reports where the level of soybean response was lower for fomesafen than for both acifluorfen and lactofen (Aulakh et al. 2016, Hager et al. 2003). Overall, findings from this study suggests that the addition of various POST herbicides may increase soybean response to dicamba tank contamination in terms of soybean visual response as evaluated variable.

Soybean plant height at 21DAA. Dicamba dose and herbicide used had significant effects on plant height at 21DAA ($P<0.0001$) which can be seen in Table 6. Based on findings evaluated POST herbicides with both 0 and 0.560 g ae ha^{-1} dicamba doses does not appear to affect soybean plant height at 21 DAA if compared with non-treated control. Plant height reduction was observed just for treatment combination which included fomesafen with 0.056 g ae ha^{-1} dicamba in tank-mixture.

Based on Pearson correlation (Table 12) findings for response variables like visual symptomology and plant height at 21 DAA were observed as negatively associated. As percent of evaluated soybean response increases plant height decreased having Pearson’s coefficient of -0.49 ($P<0.0001$). Available studies show that estimations of plant height may be used as a quick way to estimate potential impact on seed yield (Weidenhamer et al. 1989). However, if soybean exposure occurs earlier in the growing seasons final predictions on the yield may be influenced by environmental conditions or other confounding variable which may affect soybean growth and development throughout the growing season. Having enough rainfall during growing season may cause visual symptoms gradually to disappear with an ability for soybean to additionally compensate for exposure instance with an stimulation of lateral branching (Andersen et al. 2004, Conley et al. 2009).

Soybean harvest height. Analysis of response variable like height of soybean plants at harvest time after exposure to POST herbicide tank-mixed with sublethal dicamba doses resulted in two-way significant herbicide by dicamba dose interaction ($P<0.0001$). Due to complexity of the soybean response followed by various confounding variables that may affect soybean plant height throughout the growing season, the results are discussed generally as overall observed trends (Table 7). When there was no dicamba in tank-mixture most of the treatment had a slight height decrease if compared with non-treated control. The only treatment that had a positive impact on plant height was observed for crop oil concentrate and clethodim (280 g ai ha^{-1}) when applied alone. Most of the applied treatments that contained dicamba at dose of 0.056 g ae ha^{-1} resulted in slight plant height increase with impact dependent on POST herbicide used. The only herbicide for 0.056 g ae ha^{-1} dicamba dose tested and resulted in plant height decrease was associate with treatment which included fomesafen having a similar pattern across all tested dicamba doses. Using Pearson's correlation (Table 12) similar finding like ones described for plant height 21 DAA may be observed even for harvest height of soybean while percent of symptomology observed increases soybean plant harvest height decreases suggesting there might be a negative association among these two response variables. Additional observed association indicates positive association among measured soybean plant height at 21DAA with harvest plant height (Table 6). Having the higher plant height at 21 DAA it may appear that positive impact on harvest soybean height. Furthermore, as previously mentioned potentially there might be a lot of variables which may impact final soybean height and throughout this study has not been encountered for. Analysis of final plant height as response variable did not reveal any clear patterns when diverse herbicides were applied without or with dicamba in tank-mixture.

Number of pods per soybean plant and grain weight. Dataset with primary and secondary traits that might affect soybean yield for early season exposure after development of third trifoliate did not result in significant herbicide*dicamba dose interaction for number of pods developed per plant and grain weight (100 seed weight) ($P=0.177$ and $P=0.347$). Furthermore, neither for herbicide nor dicamba dose significant interaction was not observed for both response variables based on significance level of $\alpha=0.05$ level. Combining data across either dicamba doses or herbicides applied showed that there was no difference for both evaluated variables (Table 8.1, Table 8.2, Table 9.1, and Table 9.2).

Similar findings were reported in literature where number of soybean pods formed by plant and grain weight did not differ from non-treated control (Soltani et al. 2016). In order to be at least 5% of affected both number of pods per plant and grain weight the amount of dicamba in tank-mixture was estimated to be about 6.8 and 3.2 g ae ha^{-1}, respectively (Soltani et al. 2016). Even though, significant interaction was not determined based on correlation among response variables it appears that there might be positive association (Table 12) among number of plants formed per soybean plant with grain weight having coefficient of 0.243 ($P<0.0001$).

Soybean yield. Contrasts for yield of soybean exposed to POST herbicides in tank-mixtures with sublethal doses of dicamba during V3 growth stage of soybeans (Table 10) suggests that there may be a difference in terms of impact on soybean yield in comparison among untreated and treated plots for dicamba dose of 0.560 g ae ha^{-1} ($P<0.0001$). These findings support current literature findings where as dicamba dose increase in tank-mix soybean susceptibility increases. Firstly, contrasting different site of action herbicide groups results shows that difference could be determined among ACCase (group 1) and PPO (group 14) inhibiting herbicides just for base treatments that did not contained dicamba ($P=0.016$). Further, significant impact on soybean yield may be expected if compared EPSP and ACCase inhibiting herbicides just for 0.560 g ae ha^{-1} dicamba dose ($P=0.004$). Finally, when comparing EPSP and PPO inhibiting herbicides this dataset did not provide strong enough evidence to detect difference for any of the dicamba doses evaluated.

Considering all the tested herbicides according to manufacturers can be applied over the top in row crop like soybean. Findings within each dicamba dose reveals treatments which did not have dicamba as expected do not differ in terms of impact on soybean yield (Table 11.1 and Table 11.2). Even though, that numerically there is about 4% in yield decrease as dicamba dose increased in tank-mixture from 0 to 0.056 g ae ha^{-1} this data set does not provide strong enough evidence to prove difference comparing with non-treated control. In addition, after mean comparisons when dicamba was present at 0.056 g ae ha^{-1} there was a slight difference among treatments that have been applied. Confounding results related with soybean yield may be found in one of the papers available and have been associated with similar treatment combination evaluated for this study. Authors of this paper for both location used for this study there was significant yield reduction on average of 77% and 87% for dicamba treatment applied alone at 0.056 g ae ha^{-1} and an addition of COC (1% v v^{-1}), respectively (Andersen et al. 2004). Their findings suggested that visual response followed with yield if compared with non-treated control was reduced just with and addition of COC. These authors suggested as well the addition of COC potentially increased herbicide uptake from leaf surface and caused greater soybean response. Dicamba alone treatment used in this study at 0.560 g ae ha^{-1} resulted in about 13% of yield decrease with response different than both 0 g and 0.0560 g ae ha^{-1} evaluated dicamba doses.

Furthermore, noteworthy finding may be found for fluazifop-P-butyl when mixing with various dicamba doses. There was no difference observed among two simulated dicamba doses; however, significant increase of soybean yield was observed when mixed with 0.560 g ae ha^{-1} if compared with either non-treated control or when product was applied alone without the addition of dicamba. Treatments like glyphosate, setoxidim, clethodim (Intensity and Section Three), fomesafen, and COC tend to have positive impact on soybean yield if compared with treatment where dicamba was applied alone.

Consequently, as dicamba dose increased with various POST program applied over the top of soybeans difference can be observed. In contrast, treatments that outperformed treatment that contained dicamba alone was observed for fluazifop-P-butyl, clethodim (Intensity) and COC. Potential reason for soybean yield increase may be found looking at the Pearson’s correlation (Table 12) for evaluated response variables when grain weight (100 seed weight) have a positive association with soybean yield. Similar findings were reported from study that included evaluation of plant growth regulators in combination with various POST herbicides (Kelley et al. 2005). Their findings indicated that POST herbicide program can significantly intensify soybean yield response caused by tank contamination. Additional meta-analyses suggested that doses of dicamba may differ even within vegetative growth stage of soybean (Egan et al. 2014, Kniss 2018). According to Kniss (2018) dicamba dose that may cause 5% of yield drag was estimated to be about 1.9 and 5.7 g ae ha^{-1} for soybean exposure during V1 – V3 and V4 – V7 growth stage of soybean, respectively. Further, Foster and Griffin (2019) reported that yield loss of 1 and 9% was observed with dicamba doses of 0.6 and 4.4 g ae ha^{-1}, respectively. However, this data set may be used to estimate general trends what may happen to soybeans when exposed to dicamba alone through either primary or secondary off-target movement of dicamba. Considering that soybean response may be significantly affected by the addition of various herbicides in tank-mixture additional studies are needed to better understand soybean response and determine impact on future management decisions regarding with sprayer tank cleanout procedures.

Soybean exposure at reproductive (R1) growth stage. Analysis of variance (Table 4) for evaluated parameters for soybean exposed to various POST herbicide tank-mixtures with simulated sublethal dicamba doses had significant interaction between herbicide and dicamba dose ($P<0.0001$) for soybean symptomology and plant height at 21 DAA, harvest plant height, number of pods developed per soybean plant and soybean yield. Yield component such as grain weight (100 seed weight) had at the same time significant interaction for herbicide used ($P=0.001$) and simulated dicamba dose ($P<0.0001$). All presented two-way significant interactions will be discussed generally as observed trends having mean separation explanations in both ways regardless of either herbicide applied or dicamba dose used. Further, if either of simple effects were observed as significant, the mean separation will be discussed specifically how either herbicide or simulated dicamba dose impacted response variables.

Soybean symptomology at 21DAA. As presented in Table 5 general trend which can be observed as dicamba dose increase in tank-mixture soybean symptomology increases. When dicamba doses were mixed with various herbicide program there was a similarity across most of the assessed scenarios for 0 and 0.056 g ae ha^{-1}; however, 0.560 g ae ha^{-1} was a dicamba dose which intensified soybean visible response. Treatment that included dicamba alone at 0.560 g ae ha^{-1} dose resulted in 12% of visual response. Literature findings suggests that soybean exposure at R1 stage to dicamba dose at 0.75 g ae ha^{-1} resulted in 23% of visual estimation of injury (Soltani et al. 2016). Based on their findings tank contamination low as 0.75 g ae ha^{-1} considerable consequences may be expected for soybean exposed late in the season. Available meta-analysis suggests dicamba doses that was enough to cause at least 5% or crop visual response were estimated to be about 0.038 g ae ha^{-1} of the field use dose of dicamba (Kniss 2018).

The main difference in terms of visual response has been observed in present study for group 14 herbicides (PPO inhibiting herbicides). Increase of soybean response was associated when those herbicides were with dicamba dose of 0.560 g ae ha^{-1}. Treatment combinations that resulted in the greatest soybean symptomology for this study was reported 48% and it was associated with products like lactofen and fomesafen. Apart from those, application of full recommended dose of product like acifluorfen resulted in considerably lower soybean response, which may help in determination how various formulation within the same herbicide group may impact plant recovery properties.

Additionally, noteworthy relationship has been observed among various applied ACC-ase inhibiting herbicides. Application of group 1 herbicides resulted in inverse relationship for clethodim products whereas as the amount of clethodim in formulation increases visual estimation of symptoms decreases.

This phenomenon has been well described in literature as antagonistic interaction between those two chemistries but in terms of reduced efficacy associated with weed control for both grass and broadleaf troublesome weed species (Aguero-Alvarado et al. 1991, Doretto et al. 2019, Rilakovic et al. 2016, Underwood et al. 2016). Partial explanation for decreased visual appearance of symptoms may be due to deactivation of sublethal dicamba doses with a higher concentration of clethodim in the tank-mixture. To confirm this response of herbicide interaction additional analyses needs to be implemented (Colby 1967).

As reported sensitivity of soybean exposure later in growing season increases especially when soybean start with blooming (Egan et al. 2014, Kniss 2018). Considering limited number of scientific papers available for data comparison related with soybean exposure to combination of various dicamba doses with POST herbicide program at R1 growth stage was limited. The only treatment performance comparison was based on soybean exposure during V7 growth stage for herbicide interaction study that was conducted and for R2 growth stage study that they conducted to evaluate effects when plant growth regulators were applied at fraction of label dose (Kelley et al. 2005). Results presented by these authors suggested the addition of herbicide program to dicamba at both tested doses of 0.56 and 5.6 g ae ha^{-1} altered response of soybean. When applied alone for V7 and R2 growth stage soybean response was 31 and 25% for lower dicamba dose used, followed with 41 and 41% for 0.56 and 5.6 g ae ha^{-1}, respectively. Further, their findings suggested that there was an evident difference observed among products like glyphosate and fomesafen when applied alone observed soybean response was 0 and 6 % while in dicamba tank-mixed 35 and 45%, respectively. Data set provided in present study support findings that soybean symptoms may intensify with the addition of dicamba in tank-mixed with various POST management program as well as increased soybean sensitivity during reproductive stage.

Soybean plant height at 21DAA. Having complexity regarding plant response results were discussed as generally observed trends (Table 6). Results for various dicamba doses tested shows that majority of the treatments resulted in similar response. Previously published studies pointed out impact on the plant height reduction during R1 growth stage exposure has been determined rather as dicamba dose dependent. According to Griffin et al. (2013) height reduction ranged from 1 to 44% for dicamba doses from 1.1 to 70 g ae ha^{-1}, respectively. Additional literature findings shows when exposed at 16 g ae ha^{-1} impact on soybean height reduction has been reported as high as 25% if compared with non-treated control (Weidenhamer et al. 1989).

In present study the addition of various POST herbicides programs impacted response variable like plant height. Even though, most of the herbicides from group 1 (ACCase inhibitors) and 9 (EPSPS inhibitor) did not impacted soybean plant height at 21 DAA treatment, findings associated with group 14 (PPO inhibitors) were considerably distinct. Application of acifluorfen over the top of soybean did not differ from non-treated control. However, application of fomesafen and lactofen resulted in about 10% of plant height in comparison with non-treated control. The addition of 0.560 g ae ha^{-1} dicamba into tank-mix resulted with more diversified response for plant height. The highest dicamba dose used for this study reveals completely different pattern where treatment that included fomesafen resulted in significantly greater reduction if compared with all other treatments used for this study. On contrary for setoxidim at 315 g ai ha^{-1}, clethodim at 280 g ai ha^{-1}, and COC positive impact on plant height at 21 day after treatment application.

Comparisons across three tested dicamba doses shows that for Roundup Powermax there was a no difference observed; however, with Roundup Weathermax plant height was significantly affected for dicamba doses of 0 and 0.560 g ae ha^{-1}. Application of dicamba doses with ACCase inhibiting herbicides shows diversity of soybean response across tested herbicides as well as dicamba doses used. For example, for fluazifop-P-butyl there was no difference detected between doses two lower doses of dicamba, while the highest tested dose affected plant growth. Products that contained clethodim as active ingredient were no different for treatments applied at 280 g ai ha^{-1} across all tested doses of dicamba, whereas the one formulated as 272 g ai ha^{-1} had a significant impact on plant height reduction for 0.056 and 0.560 ga ae ha^{-1}. Considering unavailability of scientific papers that included POST herbicide treatments in tank-mixture with dicamba comparison with work conducted in the past was not possible.

Soybean plant height at maturity. General trend which are observed for most of the applied treatments was as dicamba dose increased in tank-mixture plant height decreased (Table 7). Overall results indicate the addition of dicamba in tank-mixture may have a significant impact on final plant height. Treatments without dicamba inside the mixture resulted in the highest plant height reduction just for PPO inhibiting herbicides having fomesafen and lactofen more responsive on soybean than acifluorfen. Further, two glyphosate products were evaluated showed significant difference among them where Roundup Weathermax did not differ from non-treated control whereas Roundup Powermax caused significant plant height reduction.

Treatments which contained dicamba at dose of 0.056 g ae ha^{-1} had a similar impact on the plant height. From all applied treatments at this dicamba dose plant height reduction was determined for fomesafen and lactofen. Similar findings have been reported for fomesafen when found with dicamba in the tank-mix having a 37% of plant height reduction (Kelley et al. 2005). In general, the highest dose of dicamba associated with various POST herbicides resulted in the highest reduction of final plant height. Treatments that had an the most significant impact on height was fomesafen applied at the full recommended dose, followed by lactofen and both glyphosate formulations used in this study. All other treatments tested for the dose of dicamba at 0.056 g ae ha^{-1} performed similarly or plant height was increased if compared with non-treated control.

Number of pods per soybean plant and seed weight. Considering complexity in soybean response after exposure to those two factors observed results will be discussed as generally observed trends (Table 8.3). In general, sublethal dicamba doses were with no difference among treatments which did not include dicamba in tank-mixture. However, simultaneously across every individual dicamba doses it appears for treatment like Roundup Powermax number of pods per plant was greater for dicamba dose at 0.056 g ae ha^{-1} in comparison with 0 g ae ha^{-1} dicamba in tank-mixture followed with no difference among 0.056 and 0.560 g ae ha^{-1}. On contrary, for application of POST herbicide like acifluorfen and fomesafen difference was confirmed. When there was no dicamba in tank-mixture number of pods created per plant if compared with non-treated control was greater for both products. The addition of both 0.056 and 0.560 g ae ha^{-1} of dicamba resulted in considerably lowered the number of pods developed per soybean plant.

Weight of 100 seed grains as can be seen from Table 9.1 and Table 9.2 combining data across dicamba doses resulted in no difference among non-treated control and Roundup Weathermax followed with all ACCase inhibiting herbicides tested. Furthermore, for all other evaluated products that were not aforementioned resulted in a significant impact on reduction of the seed weight. Combining data across herbicides reveals that there might not be difference between treatments that did not include dicamba in tank-mix versus the lowest dicamba dose that has been tested (0 and 0.056 g ae ha^{-1}). Moreover, significant difference on this response variable may be expected for treatments which included dicamba dose at 0.560 g ae ha^{-1}.

Soybean yield. Contrasts for yield of soybean exposed to POST herbicides in tank-mixtures with sublethal doses of dicamba during R1 growth stage of soybeans (Table 10) shows that there was a significant response on soybean yield in comparison among untreated and treated plots for dicamba dose of 0 and

0.056 g ae ha^{-1} (P=0.010 and P=0.011). Supplementary, contrasting different herbicide site of action groups difference was not determined among either ACCase and PPO or EPSP and PPO inhibiting herbicides just when there was no dicamba as tank contaminant while for both dicamba doses used 0.056 and 0.560 g ae ha^{-1} included difference was determined. At the same time, when comparing EPSPS and ACCase inhibiting herbicides there was no difference observed across any of the dicamba dose used.

Considering complexity of variables that may impact soybean yield general trends observed for combination of POST program with simulated dicamba doses will be discussed (Table 11.1 and Table 11.2). For treatments with 0 g ae ha^{-1} of dicamba results shows that just glyphosate (Roundup Weathermax), setoxidim , and fluazifop-P-butyl resulted in similar response as non-treated control, whereas all other evaluated treatments had a slight soybean yield decrease. For treatments which contained dicamba at 0.056 g ae ha^{-1} majority of treatments had a positive impact on soybean yield. Decrease of soybean yield for R growth stage of soybean was determined within 0.056 g ae ha^{-1} dicamba dose were associated when dicamba was applied alone and combined with products like lactofen and clethodim applied at 360 g ai ha^{-1}.

As dicamba increased in tank-mix to 0.560 g ae ha^{-1} impact of various POST herbicide program added in tank-mixture is more noted. Previously conducted studies reported with applications of 0.6, 2.2, and 4.4 g ae ha^{-1} resulted in a 2, 5, and 17% of yield reduction (Foster and Griffin 2019, Griffin et al. 2013). Available meta-analyses provided report for soybean exposure during reproductive stage suggesting yield loss of 5% may be expected with application of dicamba at of 0.9 g ae ha^{-1} (Kniss 2018). In present study the addition of POST herbicides impacted soybean yield and was associated with applied treatments like fluazifop-P-butyl, fomesafen, lactofen, and COC. Treatment combinations that resulted differently that either base treatment or non-treated control were related with products like glyphosate, setoxidim, clethodim (Section Three), and fomesafen. Setoxidim and fomesafen had a similar relationship with no difference in yield when compared across dicamba doses with non-treated control. On contrary, comparing three dicamba doses across treatment which included clethodim at 280 g ai ha^{-1} (Section Three) the highest dicamba dose tested as tank contaminant resulted in greater yield if compared non-treated control. Similar observation may be applied for glyphosate applied at 1120 g ae ha^{-1} (Roundup Powermax).

Treatment comparison within each of the tested dicamba doses reveals how various herbicide program influenced soybean yield. Interestingly, even though all the tested herbicide programs were labeled for use for POST weed control in soybeans for most of the applied treatments applied alone during R1 growth stage had an impact on final soybean yield. The soybean response for the yield as aforementioned may be significantly impacted by various environmental conditions that have been observed during 2018 and 2019 growing season.

The addition of dicamba at 0.056 g ae ha^{-1} resulted in slight yield increase for treatments that included dicamba applied alone followed by clethodim

(Section Three) and lactofen. Increasing dicamba dose in tank-mix appears that soybean yield was more affected. The level of crop response was dependent on POST herbicide applied as well on the dose of dicamba found in tank-mixture. It seems that both glyphosate products tested followed by most of the ACCase inhibiting herbicides does not have an impact on soybean yield. Further, among PPO inhibiting herbicides there are slight differences among products used where it appears after application of acifluorfen. This finding partially may be explained due to faster soybean recovery when acifluorfen was applied if compared with lactofen and fomesafen.

Results emphasize need for a more thoroughly cleanout of sprayer or if possible to have a specially designated sprayer just for dicamba applications. Identification of criteria when various POST herbicide programs were involved with a variety of dicamba doses applied over the top of sensitive soybeans may be detrimental to evaluate effects on plant response. Knowing that the sensitivity of soybeans significantly changed with an addition of herbicides, data set like this needs to be performed under multiple environments and across various varieties of soybean in order to get as much as possible diversified data set that could be used in future to build a prediction model that will help in better understanding of soybean response when exposed to diversified POST herbicide program across multiple dicamba doses through either simulated doses or sampling sprayers after dicamba application occurred to try to have as much as possible real case scenario.

List of Tables

Table 1. List of herbicides used for evaluation of non-dicamba-tolerant soybean exposure to application of postemergence herbicides in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

Active ingredient	Site of Action[a]	Trade name	Formulation g L^{-1}	Rate g ai ha^{-1} or v v^{-1}	Manufacturer
Glyphosate	EPSPS	Roundup Powermax	660	1260[b]	Bayer Crop Science
Glyphosate	EPSPS	Roundup Weathermax	660	1260[b]	Bayer Crop Science
Setoxidim	ACCase	Poast Plus	120	315[c]	BASF
Fluazifop-P-butyl	ACCase	Fusilade DX	240	210[c]	Syngenta
Clethodim	ACCase	SelectMax	116	272[c]	Valent
Clethodim	ACCase	Intensity	240	280[c]	Loveland
Clethodim	ACCase	Section Three	360	280[c]	Winfield
Aciflurofen	PPO	Ultra Blazer	240	420[d]	UPL
Fomesafen	PPO	Flexstar	225	530[d]	Syngenta
Lactofen	PPO	Cobra	240	220[d]	Valent
Crop oil concentrate[d]		R.O.C.		1%	Wilbur-Ellis

[a] Abbreviations used for herbicide site of action: EPSPS, enolpyruvylshikimate-3-phosphate synthase (group 9); ACCase, Acetyl CoA Carboxylase (group 1); PPO, protoporphyrinogen oxidase (group 14).

[b] Ammonium sulfate 20 g L-1 (Bronc, Wilbur-Ellis).

[c] Non-ionic surfactant 0.25% v v^{-1} (R-11 Spreader Activator, Wilbur-Ellis).

[d] Crop oil concentrate 1% v v^{-1} (R.O.C., Wilbur-Ellis).

Dicamba (XtendiMax, Bayer Crop Science) all treatments included three different dicamba doses as tank contaminants 0, 0.056, 0.560 g ae ha^{-1} which represents 0, 0.01%, and 0.1% of standard recommended labeled dose of 560 g ae ha^{-1}.

Table 2. Environmental conditions for application of treatments over the top of soybean for 2018 and 2019 growing season.

	V3		R1	
	2018	2019	2018	2019
Date	06/23/2018	06/24/2019	07/09/2018	07/09/19
Wind speed	W 2.45 m s^{-1}	W 1.6 m s^{-1}	E 2.32 m s^{-1}	W 2.62 m s^{-1}
Air temperature	26 ˚C	22 ˚C	28 ˚C	24 ˚C
Humidity	45%	66%	43%	70%

Table 3. Monthly rain precipitation and temperature at North Platte, NE, in 2018 and 2019[a].

	Rainfall		Temperature	
Month	2018	2019	2018	2019
	mm		°C	
April	28.64	39.27	5.45	9.18
May[b]	173.30	163.63	16.83	11.53
June	109.06	81.83	21.50	19.41
July	129.62	175.41	22.76	23.68
August	7.78	93.81	21.26	21.71
September	6.86	26.01	18.49	20.12
October[c]	45.01	14.99	8.35	5.98
Total[d]	500.27	594.95	114.64	111.61

[a] Data obtained from National Weather Service (https://www.weather.gov/).

[b] Planting month.

[c] The harvest month.

[d] Total precipitation from planting until harvest.

Table 4. Analysis of variance for evaluated parameters on soybean exposed to postemergence herbicides in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

	V3 growth stage											
Factor	Symptoms 21 DAA[a]		Height 21 DAA		Harvest height		Pods		100 Seed weight		Yield	
	Fc	ρ-value	Fc[b]	ρ-value	Fc	ρ-value	Fc	ρ-value	Fc	ρ-value	Fc	ρ-value
Herbicide (H)	94.3	<0.0001	4.3	<0.0001	8.6	<0.0001	1.1	0.345	1.0	0.442	2.4	0.007
Dicamba dose (D)	2819.1	<0.0001	6.4	<0.0001	43.2	<0.0001	0.4	0.664	2.5	0.083	1.0	0.375
H x D	20.5	<0.0001	2.9	<0.0001	4.0	<0.0001	1.3	0.177	1.1	0.347	2.4	<0.0001
CV[c] (%)	22.42		6.32		2.94		16.29		2.98		7.04	

	R1 growth stage											
Factor	Symptoms 21 DAA		Height 21 DAA		Harvest height		Pods		100 Seed weight		Yield	
	Fc	ρ-value	Fc	ρ-value	Fc	ρ-value	Fc	ρ-value	Fc	ρ-value	Fc	ρ-value
Herbicide (H)	95.6	<0.0001	11.6	<0.0001	14.3	<0.0001	2.7	0.003	3.0	0.001	2.6	0.004
Dicamba dose (D)	1333.4	<0.0001	6.3	<0.0001	77.7	<0.0001	0.6	0.527	20.5	<0.0001	2.8	0.062
H x D	8.4	<0.0001	2.6	<0.0001	2.7	<0.0001	2.5	<0.0001	0.9	0.466	2.7	<0.0001
CV (%)	29.99		4.89		2.72		17.90		3.24		6.86	

[a] Days After Application.

[b] Fc: Calculated F-value.

[c] CV: Coefficient of Variance.

Table 5. Visual estimation on soybean symptomology at 21 days after application of postemergence herbicides in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

Herbicide	Dicamba dose[a] (g ae ha^{-1})					
	V3 growth stage			R1 growth stage		
	0	0.056	0.560	0	0.056	0.560
	%					
Non-treated	0.0 aA	1.1 aA	9.4 bA	0.0 aA	2.5 aA	11.8 bA
Roundup Powermax	0.0 aA	1.9 aA	41.0 bD	0.6 aA	1.0 aA	29.1 bD
Roundup Weathermax	0.0 aA	1.9 aA	40.6 bD	0.0 aA	1.6 aA	29.1 bD
Poast Plus	0.0 aA	0.4 aA	38.8 bD	0.0 aA	3.2 aA	35.3 bE
Fusilade DX	0.0 aA	2.3 aA	40.4 bD	0.0 aA	1.2 aA	27.0 bC
SelectMax	0.0 aA	1.8 aA	40.8 bD	0.0 aA	2.5 aA	30.8 bD
Intensity	0.0 aA	0.6 aA	30.6 bB	0.0 aA	1.2 aA	26.0 bC
Section Three	0.6 aA	1.8 aA	31.6 bB	0.0 aA	0.0 aA	22.0 bB
Ultra Blazer	11.3 aB	16.3 bB	36.9 cC	9.3 aB	9.3 aB	25.7 bC
Flexstar	15.0 aC	18.9 aB	49.9 bE	16.0 aC	11.8 aB	48.0 bF
Cobra	18.1 aC	16.6 aB	43.8 bE	25.3 aD	24.2 aC	47.5 bF
COC[b]	0.0 aA	0.0 aA	34.1 bC	0.0 aA	1.8 aA	29.1 bD

[a] Means followed by the same letter, lower case in the row within growth stage and upper case in the column, do not differ using Tukey and Scott Knott's tests, respectively, at $\alpha = 0.05$.

[b] Crop Oil Concentrate.

Table 6. Height of soybean plants at 21 days after application of postemergence herbicides in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

Herbicide	Dicamba dose[a] (g ae ha^{-1})					
	V3 growth stage			R1 growth stage		
	0	0.056	0.560	0	0.056	0.560
	cm					
Non-treated	25.0 ab	26.3 bC	24.4 a	58.7 B	59.0 C	57.9 C
Roundup Powermax	25.2 ab	26.9 bC	23.6 a	58.4 B	57.7 C	56.7 C
Roundup Weathermax	25.3	25.8 C	24.3	58.5 bB	58.2 abC	55.1 aB
Poast Plus	24.5	23.9 B	24.2	56.7 aB	60.9 bC	58.9 abD
Fusilade DX	25.0	25.5 C	25.2	60.1 bB	60.0 bC	56.3 aC
SelectMax	25.0	24.7 C	23.3	59.2 bB	55.5 aB	56.9 abC
Intensity	26.2 b	24.7 abC	24.1 a	59.7 B	60.4 C	57.4 C
Section Three	26.5	25.5 C	24.8	59.6 B	59.4 C	60.7 D
Ultra Blazer	24.3	23.8 B	25.2	56.4 abB	59.4 bC	55.6 aB
Flexstar	24.1 b	21.4 aA	24.3 b	52.6 aA	57.4 bC	51.9 aA
Cobra	23.9	24.1 B	24.1	53.2 A	52.9 A	54.4 B
COC[b]	25.3 ab	25.8 bC	23.6 a	58.3 B	56.8 B	58.6 D

[a] Means followed by the same letter, lower case in the row within growth stage and upper case in the column, do not differ using Tukey and Scott Knott's tests, respectively, at α = 0.05.

[b] Crop Oil Concentrate.

Table 7. Height of soybean plants at harvest after exposure to postemergence herbicides in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

Herbicide	Dicamba dose[a] (g ae ha^{-1})					
	V3 growth stage			R1 growth stage		
	0	0.056	0.560	0	0.056	0.560
	cm					
Non-treated	82.6 bB	82.7 bC	79.8 aB	80.6 bC	77.6 aB	76.5 aC
Roundup Powermax	80.8 aA	83.9 bC	78.8 aB	78.9 bB	79.0 bB	73.7 aB
Roundup Weathermax	79.4 aA	83.4 bC	77.2 aA	81.1 cC	77.8 bB	73.4 aB
Poast Plus	78.5 A	80.6 B	80.0 B	80.3 bC	78.7 bB	75.7 aC
Fusilade DX	82.5 bB	83.1 bC	79.0 aB	79.5 bC	79.5 bB	75.6 aC
SelectMax	83.8 bB	81.2 bB	76.9 aA	78.0 B	79.6 B	77.8 C
Intensity	85.4 cC	81.9 bC	78.8 aB	79.5 bC	80.7 bB	76.5 aC
Section Three	80.1 A	81.5 B	79.7 B	77.8 B	79.9 B	77.4 C
Ultra Blazer	79.5 abA	80.4 bB	77.4 aA	78.1 abB	79.0 bB	76.5 aC
Flexstar	78.0 A	77.8 A	78.6 B	75.1 bA	75.7 bA	70.9 aA
Cobra	79.2 A	80.1 B	80.0 B	75.5 bA	75.1 abA	72.6 aB
COC[b]	84.8 bC	85.2 bC	80.0 aB	79.2 abB	79.9 bB	76.8 aC

[a] Means followed by the same letter, lower case in the row within growth stage and upper case in the column, do not differ using Tukey and Scott Knott's tests, respectively, at α = 0.05.

[b] Crop Oil Concentrate.

Table 8.1. Number of pods per soybean plant exposed to postemergence herbicides at V3 growth stage [a].

Herbicide	Pods plant^{-1}
Non-treated	62
Roundup Powermax	69
Roundup Weathermax	72
Poast Plus	67
Fusilade DX	66
SelectMax	67
Intensity	66
Section Three	69
Ultra Blazer	65
Flexstar	67
Cobra	66
COC[b]	69

[a] There were no differences using Scott Knott's test at $\alpha = 0.05$. Data combined across dicamba doses.

[b] Crop Oil Concentrate.

Table 8.2. Number of pods per soybean plant exposed to sublethal doses of dicamba at V3 growth stage.

Dicamba dose	Pods plant^{-1}
g ae ha^{-1}	
0	66.9
0.056	66.5
0.560	67.9

There were no differences using Tukey's test at $\alpha = 0.05$. Data combined across herbicides.

Table 8.3. Number of pods per soybean plant exposed to postemergence herbicides in tank-mixtures with sublethal doses of dicamba at R1 growth stage.

Herbicide	Dicamba dose[a] (g ae ha^{-1})		
	0	0.056	0.560
	pods plant^{-1}		
Non-treated	58 A	60 A	57 A
Roundup Powermax	56 aA	71 bB	63 abA
Roundup Weathermax	63 B	57 A	60 A
Poast Plus	63 aB	65 abB	76 bB
Fusilade DX	63 B	63 B	62 A
SelectMax	50 A	61 A	54 A
Intensity	55 A	55 A	62 A
Section Three	60 A	59 A	67 B
Ultra Blazer	65 B	55 A	61 A
Flexstar	75 bB	53 aA	51 aA
Cobra	58 A	64 B	68 B
COC[b]	55 A	55 A	57 A

[a] Means followed by the same letter, lower case in the row within growth stage and upper case in the column, do not differ using Tukey and Scott Knott's tests, respectively, at α = 0.05.

[b] Crop Oil Concentrate.

Table 9.1. Weight of 100 soybean seeds exposed to postemergence herbicides at V3 and R1 growth stages.

Herbicide	Soybean growth stage		
	V3	R1	
	g 100 grains^{-1}		
Non-treated	16.48	16.32	B
Roundup Powermax	16.57	15.88	A
Roundup Weathermax	16.35	16.12	B
Poast Plus	16.23	16.02	B
Fusilade DX	16.54	16.20	B
SelectMax	16.34	16.20	B
Intensity	16.53	16.06	B
Section Three	16.52	15.94	A
Ultra Blazer	16.42	15.90	A
Flexstar	16.46	15.77	A
Cobra	16.48	15.70	A
COC[b]	16.50	15.99	A

[a] Means followed by the same letter do not differ using Scott Knott's tests, at α = 0.05. Data combined across dicamba doses.

[b] Crop Oil Concentrate.

Table 9.2. Weight of 100 soybean seeds exposed to sublethal doses of dicamba at V3 and R1 growth stages.

Dicamba dose	Soybean growth stage		
	V3	R1	
g ae ha^{-1}	g 100 seeds^{-1}		
0	16.51	16.15	B
0.056	16.49	16.15	B
0.560	16.37	15.76	A

Means followed by the same letter in the column do not differ using Tukey's test at α = 0.05. Data combined across herbicides.

Table 10. Contrasts for yield of soybean exposed to postemergence herbicides in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

Contrast	Dicamba dose (g ae ha^{-1})					
	V3 growth stage			R1 growth stage		
	0	0.056	0.560	0	0.056	0.560
	ρ-value			ρ-value		
Untreated vs Treated	0.364	0.182	<0.0001	0.010	0.011	0.796
ACCase vs PPO	0.016	0.090	0.087	0.378	0.011	0.039
EPSPs vs PPO	0.076	0.326	0.191	0.296	0.051	0.006
EPSPs vs ACCase	0.860	0.680	0.004	0.710	0.926	0.221

Table 11.1. Yield of soybean exposed to postemergence herbicides in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

Herbicide	Dicamba dose[a] (g ae ha^{-1})					
	V3 growth stage			R1 growth stage		
	0	0.056	0.560	0	0.056	0.560
	kg ha^{-1}					
Non-treated	5537 b	5289 bA	4835 aA	5536 bB	5000 aA	5161 abA
Roundup Powermax	5386 ab	5667 bB	5141 aA	4919 aA	5344 bB	5437 bB
Roundup Weathermax	5535	5282 A	5250 A	5587 B	5440 B	5314 B
Poast Plus	5319	5661 B	5601 B	5393 abB	5470 bB	4999 aA
Fusilade DX	5210 a	5354 abA	5753 bB	5403 B	5443 B	5057 A
SelectMax	5666	5430 A	5230 A	5080 A	5335 B	5253 B
Intensity	5592	5497 B	5701 B	5180 A	5566 B	5423 B
Section Three	5616	5664 B	5347 A	5008 aA	5195 abA	5493 bB
Ultra Blazer	5311	5226 A	5380 A	5118 A	5246 B	5408 B
Flexstar	5135	5558 B	5267 A	5164 abA	5290 bB	4854 aA
Cobra	5277	5276 A	5424 A	5111 A	4957 A	4895 A
COC[b]	5447	5633 B	5680 B	5129 A	5464 B	5063 A

[a] Means followed by the same letter, lower case in the row within growth stage and upper case in the column, do not differ using Tukey and Scott Knott's tests, respectively, at α = 0.05.

[b] Crop Oil Concentrate.

Table 11.2. Soybean yield as percentage of non-treated control for soybean exposure to postemergence in tank-mixtures with sublethal doses of dicamba at V3 and R1 growth stages.

Herbicide	Dicamba dose[a] (g ae ha^{-1})					
	V3 growth stage			R1 growth stage		
	0	0.056	0.560	0	0.056	0.560
	%					
Non-treated	100 b	96 bA	87 aA	100 bB	90 aA	93 abA
Roundup Powermax	97 ab	102 bB	93 aA	89 aA	97 bB	98 bB
Roundup Weathermax	100	95 A	95 A	101 B	98 B	96 B
Poast Plus	96	102 B	101 B	97 abB	99 bB	90 aA
Fusilade DX	94 a	97 abA	104 bB	98 B	98 B	91 A
SelectMax	102	98 A	94 A	92 A	96 B	95 B
Intensity	101	99 B	103 B	94 A	101 B	98 B
Section Three	101	102 B	97 A	90 aA	94 abA	99 bB
Ultra Blazer	96	94 A	97 A	92 A	95 B	98 B
Flexstar	93	100 B	95 A	93 abA	96 bB	88 aA
Cobra	95	95 A	98 A	92 A	90 A	88 A
COC[b]	98	102 B	103 B	93 A	99 B	91 A

[a] Means followed by the same letter, lower case in the row within growth stage and upper case in the column, do not differ using Tukey and Scott Knott's tests, respectively, at α = 0.05.

[b] Crop Oil Concentrate.

Table 12. Pearson correlation for evaluated parameters on soybean exposed to postemergence herbicides tank-mixed with sublethal doses of dicamba at V3 and R1 growth stages.

Parameter 1	Parameter 2	V3 growth stage		R1 growth stage	
		Pearson's coefficient	ρ-value	Pearson's coefficient	ρ-value
Injury	Height 21DAA	-0.490	<0.0001	-0.095	0.106
	Harvest height	-0.490	<0.0001	-0.316	<0.0001
	Pods plant^{-1}	0.041	0.493	0.047	0.424
	100-grain weight	-0.112	0.057	-0.418	<0.0001
	Yield	-0.082	0.167	-0.189	0.001
Height 21DAA	Harvest height	0.360	<0.0001	0.893	<0.0001
	Pods plant^{-1}	0.074	0.210	-0.497	<0.0001
	100-grain weight	-0.049	0.406	-0.201	0.001
	Yield	0.107	0.070	0.014	0.815
Harvest height	Pods plant^{-1}	-0.041	0.490	-0.432	<0.0001
	100-grain weight	-0.031	0.601	-0.084	0.155
	Yield	0.110	0.063	0.073	0.217
Pods plant^{-1}	100-grain weight	0.243	<0.0001	0.170	0.004
	Yield	0.057	0.337	0.039	0.515
100-grain weight	Yield	0.244	<0.0001	0.052	0.379

Supplemental List of Tables

Table S1. Normality of residuals and homogeneity of variance of data.

			Original data		Transformed data			
Stage	Parameter	Test	*F/KS values*	Significance	Type of transfomation	*F/KS values*	Significance	Analysis
V3	Visual 21 DAA	Levene	4.634	0.000	asin(√x/100)	8.276	<0.0001	NT
		KS	0.115	0.000		0.132	<0.0001	
	Height 21DAA	Levene	2.387	0.000	rootsquare(x)	2.204	<0.0001	NT
		KS	0.026	0.200		0.031	0.200	
	Harvest Height	Levene	2.364	0.000	rootsquare(x)	2.612	<0.0001	NT
		KS	0.040	0.200		0.048	0.200	
	Number of pods per plant	Levene	1.230	0.185	-			NT
		KS	0.054	0.043				
	Grain weight	Levene	1.093	0.339	-			NT
		KS	0.035	0.200				
	Yield	Levene	0.961	0.537	-			NT
		KS	0.054	0.044				
R1	Visual 21 DAA	Levene	7.257	0.000	asin(√x/100)	8.585	<0.0001	NT
		KS	0.143	0.000		0.140	<0.0001	
	Height 21DAA	Levene	1.342	0.104	-			NT
		KS	0.038	0.200				
	Harvest Height	Levene	1.771	0.007	rootsquare(x)	1.741	0.009	NT
		KS	0.032	0.200		0.032	0.200	
	Number of pods per plant	Levene	2.204	0.000	rootsquare(x)	2.129	<0.0001	NT
		KS	0.054	0.039		0.036	0.200	
	Grain weight	Levene	0.716	0.883	-			NT
		KS	0.042	0.200				
	Yield	Levene	1.490	0.044	-			NT
		KS	0.041	0.200				

NT: non-transformed

List of Figures

Figure 2.1. Experimental unit (6 rows plot) organization in the field with row use explanation.

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CHAPTER 3

Effects of Drift-Reducing Nozzles and Agents on Dicamba Efficacy

Abstract

The increase in cropping area with dicamba-tolerant crops in the USA was followed with increased number reported cases of off-target movement (OTM). The addition of drift-reducing agents (DRAs) with certain tank-mixtures is required along with drift-reducing nozzle types. Impact of these techniques on application process and weed control is not well understood. The objectives of this study were to evaluate the impact of DRAs added to dicamba tank-mixtures on droplet size and weed control. Droplet size distribution (DSD) was impacted by nozzle type, solution, and operating pressure. Across all tested pressures DSD values followed pattern with TTI>TDXL-D>ULD (largest to smallest) with significant decrease in OTM potential observed when DRAs are added. Applications of dicamba with guar gum (DRA 1) at 138 kPa resulted in pattern collapse for TDXL-D and ULD nozzle types. Increasing the operational pressure to 207 and 276 kPa overcame pattern collapse issues observed for TDXL-D and ULD nozzles. Velvetleaf biomass reduction was 94% of greater. However, for weeds with hard to wet surface, like one on common lambsquarters, biomass reduction ranging from 74 to 87%, with plant position relative to nozzle and treatment used being significant factors in the biomass reduction measured. Minimization of OTM is a priority, there is a critical need to determine which label approved mitigation practices are most effective and which ones may be detrimental to extend lifetime of chemistry use and optimize weed control.

Introduction

Management of undesirable plants in row crops represents a critical component for sustainable food production. Various herbicides have been used as an important tool to reduce or completely remove weed competition in row crops. Low cost of chemicals and labor requirements, quick response, and satisfactory control has led to the overreliance on herbicides applied on crops. As a negative impact, continuous use of herbicides with the same or similar mode of action has led to the evolution of weed resistance substantially shifting the weed communities found in row crops (Heap 2014, Vencill et al. 2012). Resistant populations of weeds have been confirmed to various herbicides (Tranel and Wright 2002, Vieira et al. 2017, Vieira et al. 2018, Jhala et al. 2014). Considering limitations on currently available herbicides for postemergence (POST) weed control, additional tools in row crop weed control are needed to decrease the evolution of weed resistance. A major advance in agricultural production was the development and commercialization of herbicide-tolerant crops have originated a tremendous change in agriculture by providing alternatives and highly effective methods for weed management to growers (Dodson 2019, Green 2009, Kniss 2018).

Dicamba-tolerant (DT) crops were introduced during 2016 growing season as an alternative to integrate more diverse herbicide options in row crop weed control (Taylor et al. 2017). As a result of flexible application timing for late control of emerging weeds, the DT cropping system is being widely accepted. In Nebraska alone, 19%, 39%, and 52% of the entire planted area with soybeans were cultivated with DT cultivars in 2017, 2018, and 2019 growing seasons, respectively (USDA 2018, USDA NASS 2019, USDA NASS 2020, Werle et al. 2018). With the continuous adoption of DT crops over the last several years an increase in dicamba products use for broadleaf weed control through the USA is expected (USGS 2020).

Even though, the introduction of DT crops provided growers an effective site of action for weed control, there are several concerns associated with off-target movement (OTM) of this chemistry. Unintended movement of dicamba typically has been reported due to spray particle drift, volatility, and tank contamination (Alves et al. 2017b, Riter et al. 2020, Sall et al. 2020, Soltani et al. 2016). With numerous dicamba-sensitive vegetation and crops surrounding agricultural landscapes, drift-reduction techniques are fundamental when spraying dicamba to reduce OTM. Environmental conditions, proper selection of product formulation, nozzle type and orifice size, operational pressure and speed, boom height, and tank-mix additives are some of many factors which can decrease OTM potential. The development and release of DT crops came along with new dicamba formulations and application restrictions, especially related to coarser droplet size, and use of drift-reducing agents (DRAs) (Anonymous 2018, Anonymous 2018, Anonymous 2018, Anonymous 2019).

In general, a considerable reduction in pesticide downwind deposition is expected when droplet size is increased (Alves et al. 2017b). Previous research has shown with droplet size increase lower pesticide on-target deposition and efficacy is expected (Butts et al. 2018, Creech et al. 2016, Smith et al. 2000, Wolf

2002). It has been reported that dicamba efficacy can be affected by the interactions of droplet size, carrier volume, and weed species (Sanyal et al. 2006, Butts et al. 2018, and Creech et al. 2016). The addition of tank-mix additives (DRAs) may provide enhance performance where these combinations result in decreased efficacy. The development and release of DT crops was followed with introduction of diversified DRAs for use with dicamba application. Even though, the use of DRAs are typically associated with drift mitigation, they may offer additional improvements in overall performance of a given application having their purpose often cross listed with other functions.

With the inclusion of multiple additives to a tank-mixture it is expected to result in the change of spray characteristics (Oliveira et al. 2015, Prokop and Kejklicek 2002). This typically includes changes in physical properties of given solution including density, viscosity, surface tension, evaporation time, which turn results in changes of the droplet size distribution (DSD) produced (Moraes et al. 2019). It is noted that the exiting spray fan angle may be impacted for certain nozzle types by operating pressure, nozzle design, and physical chemical properties of the spray solution (Dorr et al. 2013, Spanoghe et al. 2007).

Increased awareness of pesticides impact on environment has been reported in literature even at low doses (Relyea 2005). As a preventive measure to decrease negative impact on environmental regulatory agencies trends toward more strict guidelines for both manufactures and pesticide applicators which must adhere tend to be more restrictive than what may be required. Typically, mitigation practices meeting regulatory agencies demands are required for pesticide application especially for chemistries with high risk for OTM. Following all requirements decrease OTM and adverse environmental impact. However, the interaction of these factors within any given pesticide application is a highly complex process which includes a lot of variables that may impact both application process and treatment performance (Ebert et al. 1999, Grisso et al. 1989) where information and guidance on these interactions often lack. When performance of given application is affected by various decisions, significant impact on weed selection pressure and decrease of the lifetime use of available chemistries may occur (Bish and Bradley 2017, Vieira et al. 2020). Therefore, additional research is needed considering that there is a lack of information on how DRAs affect weed control and pesticide application effectiveness. The objectives of this study were to investigate how by label approved tank-mixed DRAs with dicamba applied using three different nozzle types and three operating pressures on droplet size and weed control.

Materials and Methods

Greenhouse and laboratory studies were conducted at the Pesticide Application Technology Laboratory of the University of Nebraska-Lincoln in North Platte, Nebraska, USA in 2019 and 2020.

Droplet size. The experiment was conducted twice in a randomized complete block with factorial arrangement of treatments (4x3x3) with three replications. Treatment factors included four solution, three nozzle types, and three operating pressures. Solution was consisted of water, dicamba - diglycolamine salt (XTM -

XtendiMax® with VaporGrip® Technology, Monsanto, St. Louis, MO, USA) applied at 560 g ae ha^{-1} either alone or in tank-mixture with two DRAs at 0.5% v v^{-1}. DRAs used were polyethylene glycol, choline chloride, guar gum (DRA 1 - Intact™, Precision Laboratories, LLC, Waukegan, IL, USA) and 2-hydroxypropane-1,2,3 carboxylate, complex trihydric alcohols, oligomeric sugar alcohol condensates (DRA 2 - Trapline™-Pro II, CHS Inc. Inver Grove Heights, MN, USA). Further, application was performed to deliver 140 L ha^{-1} using TTI 11004 (TTI - Turbo TeeJet Induction, Spraying Systems Co., Wheaton, IL, USA), TDXL 11004-D (TDXL-D - TurboDrop XL Medium Pressure D Version, Greenleaf Technologies, Covington, LA, USA), and ULD 12004 (ULD - Ultra Lo-Drift, Pentair, Minneapolis, MN, USA) nozzles with operated pressure of 138, 207, and 276 kPa. The analysis of DSD for each treatment evaluated for this study was measured using laser diffraction instrument in a low speed wind tunnel with a constant wind speed of 6.7 m s^{-1}. An individual replication for each treatment was consisted of a full traverse of the spray plume through the measurement area. More information about procedures and low speed wind tunnel set up and operation are described by Creech et al. (2016), Alves et al. (2017a), and Vieira et al. (2018). Recorded values included $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ the droplet diameters (μm) such that 10, 50, and 90% of the total spray volume is in droplets of lesser diameter, respectively. Additionally, Driftable Fines (DF - the percentage of the total spray volume consisting of droplet diameters 200 μm or less) and relative span (RS) were recorded. Relative span represents a dimensionless parameter that shows uniformity of spray distribution, calculated using an equation 1:

$$RS = \frac{DV_{0.9} - DV_{0.1}}{DV_{0.5}} \qquad [1]$$

The dataset was subjected to analysis of variance using a generalized linear mixed model (PROC GLIMMIX) in SAS (Statistical Analysis Software, version 9.4, Cary, NC, USA). All comparisons were performed at α = 0.05 significance using a Fisher's Protected LSD test. The spray classifications were based on curves from reference nozzles spraying water alone in accordance with ASABE S572.1 standard (ASABE, 2017).

Plant material. Velvetleaf (*Abutilon theophrasti* Medik.) and common lambsquarters (*Chenopodium album* L.) were planted into cone pots filled with Pro-Mix BX5 (Premier Tech Horticulture Ltd, Rivière-du-Loup, Canada) general purpose growing medium. Plants were grown under controlled greenhouse conditions with a daytime temperature 26 – 28°C and a night temperature 18 – 22°C. Supplemental LED light of 520 µmol s^{-1} (Philips Lighting, Somerset, NJ, USA) was used to extend daylight period to 16 hours. 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} blended with water. Plants were treated when they were 10 to 15 cm in height.

Spray pattern study. Greenhouse trials were conducted in a 3 x 3 x 3 split-split-plot arrangement in a randomized complete block design with four replications and three experimental runs. Previously described factors solution, pressure, and nozzle type were considered as main plot, sub-plot, and sub-sub-plot, respectively. Applications were made using a three-nozzle track spray chamber (DeVries, Hollandale, MN, USA) with nozzles spaced 50 cm apart and 50 cm above target calibrated to deliver 140 L ha^{-1} at 138, 207, and 276 kPa at 2.5 m s^{-1}, 3.0 m s^{-1}, and 3.5 m s^{-1}, respectively. Prior to applications, twelve plants of each weed species per replication were arranged in a continuous line across width of the spray boom (Figure 1). Plants were divided into two groups (variables) corresponding to plant position in relation to the nozzles: between and underneath the nozzles. After applications, plants were transferred to the greenhouse where they were kept until harvest date. Plants aboveground biomass were harvested 28 days after application (DAA) and dried at 65 °C to a constant weight. Dry biomass weights were recorded and converted into percentage of biomass reduction compared to non-treated control using an equation 2 (in which NT represents the mean biomass of non-treated plants and T represents the biomass of the treated plants):

$$\% \ Biomass\ reduction = \left[\frac{(NT - T)}{NT}\right] * 100 \qquad [2]$$

Data was subjected to joint analysis using Sisvar Statistical Software, version 5.6 (Ferreira, 2011) and combined across runs as the ratio between the highest and lowest Root Square Mean Error (RSME) was smaller than 3 (data now shown) (Box, 1954). Normality of residuals and homogeneity of variance of data were analyzed by the Kolmogorov-Smirnov and Levene's tests (Table S1), respectively, using SPSS Statistical Software, version 20 (SPSS Inc., Chicago, IL, USA). Transformation of data was not necessary at $\alpha = 0.01$ significance. Data was subjected to analysis of variance (ANOVA) using Sisvar Statistical Software, version 5.6, and comparisons were made using Tukey's mean separation test at $\alpha = 0.05$ significance.

Dose response study. Common lambsquarters plants were sprayed with dicamba (XTM - XtendiMax® with VaporGrip® Technology, Monsanto, St. Louis, MO, USA) doses of 1.1, 2.2, 4.4, 8.8, 17.5, 35, 70, 140, 280, 560, 1120 g ae ha^{-1} alone and in tank-mixture with DRA 1 or DRA 2 at 0.5% v v^{-1}. The experiment was conducted twice in a randomized complete block design with 10 replications (experimental units) per treatment. Applications were made using a single nozzle research sprayer (DeVries, Hollandale, MN, USA) calibrated to deliver 140 L ha^{-1} at 276 kPa using AI9502EVS nozzle (Air Induction, Spraying Systems Co., Wheaton, IL, USA). After applications, plants were transferred to the greenhouse where they were kept until harvest date. Aboveground plant biomass was harvested at 28 DAA and dried at 65 °C to a constant weight. Dry biomass weights were recorded and converted into percentage of biomass reduction compared to non-treated control using an equation 2. A non-linear regression, log logistic model was fitted to the data using the *DRC* package (Streibig 1980) in R software (R Foundation for Statistical Computing, Vienna, Austria) using an equation 3:

$$y = c + \{d - c/1 + \exp [\, b\ (\log x - \log e)]\} \qquad [3]$$

in which *y* corresponds to the biomass reduction (%), *b* is the slope at the inflection point, *c* is the lower limit of model, *d* is the upper limit, *x* is dicamba dose used (g ae ha^{-1}), and *e* is the inflection point (ED_{50} – the effective dose to reduce 50% of plant biomass).

Results

Droplet size study. Significant interaction for solution by pressure by nozzle were observed influencing the $Dv_{0.1}$, $Dv_{0.5}$, $Dv_{0.9}$, RS, and driftable fines (Table 1). As can be seen in Tables 2.1 through 2.3 $Dv_{0.1}$, $Dv_{0.5}$, $Dv_{0.9}$, RS, and DF presented confirms that there were significant differences in droplet size between each nozzle, pressure and solution type, which was expected based on many other previous studies. Given this, it was also expected, and supported by the data, that all exploited interactions were significant. All treatments (solutions*nozzle types*operating pressures combinations) tested resulted in ultra-course (UC) spray classification in accordance with ASABE S572.1 standard. Even with this spray classification, there are unique trends observed for each nozzle type.

Overall, the largest droplet size spray was observed with the TDXL-D nozzle operating at the lowest pressure (138 kPa) for the dicamba plus DRA 1 solution with observed $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ values of 674, 1215, and 1718 µm, respectively, though $Dv_{0.1}$ was not significantly different from the TTI nozzle using the same solution. While DRA 1 resulted in the largest overall droplet size across the four solutions for both the TTI and TDXL-D, the largest droplet size spray resulted from DRA 2 among the four solutions tested with the ULD nozzle. Generally, across all nozzle, pressure and solution combinations tested, the ULD nozzle resulted in the lowest $Dv_{0.1}$, $Dv_{0.5}$, $Dv_{0.9}$ values and the greatest percentage of driftable fines, likely as result of the different design structure of this nozzle.

In comparison with dicamba alone treatment, the addition of DRAs decreased OTM movement potential with the DRA 1 being more effective than the DRA 2 for the TDXL-D and ULD nozzles with no difference observed among DRAs for the TTI nozzle. Across all nozzle, pressure, solution combinations tested, the TDXL-D at the lowest pressure and spraying dicamba plus DRA 1 created the smallest fraction of driftable fines (0.05%). Visual observations of the spray fan revealed that with both the TDXL-D and ULD nozzles the spray fan lacked proper development when spraying the high viscous, DRAs containing solutions at low pressures. Surprisingly, water-alone resulted in the lowest droplet size data across all treatments combinations, with the exception of the TDXL-D nozzle at all three pressures, for which the dicamba solution resulted in the smallest droplet size between the four solutions tested (Table 2.1 - 2.3).

As the pressure increased from 138 to 207 kPa, the $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ in general values decreased (Table 2.2). As observed previously the addition of DRA 1 had the greatest impact on droplet size parameters increase for majority of the nozzles (Table 2.2). Impact of tank-mixtures on $DV_{0.1}$ value followed the similar trend from highest to lowest: dicamba + DRA 1> dicamba + DRA 2> dicamba across nozzle types. For applications with the TTI nozzle at 207 kPa nozzle TTI for solution with DRA 1 in tank-mixture resulted in the greatest $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ values of 620 µm, 1113 µm, and 1552 µm, respectively. Dicamba treatment applied at 207 kPa followed the same scenario as one described for 138 kPa for TTI and ULD nozzle where droplet size increased. Further, application of dicamba alone treatment using a TTI nozzle at 207 kPa resulted in 0.42 per cent of fines generated compared to applications with water where observed percentage of driftable fines were 0.60%. On contrary, for TDXL-D nozzle inverse response was determined where the addition of dicamba increased per cent of DF values. The addition of DRAs in dicamba tank-mixture, particularly for TDXL-D, was effective in reducing the percent of driftable fines for both DRA 1 and DRA 2, respectively. Findings regarding ULD nozzle follow the similar trend as observed for the lowest pressure used in this study. Across all solutions droplets generated using the ULD nozzle type were in general smaller and the percentage of fines was considerably higher if compared with the other two nozzle types.

Droplet size distribution for applications made at 276 kPa resulted in greatest decrease of the $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ (Table 2.3). Across solutions $Dv_{0.5}$ values were affected with the addition of DRAs in dicamba tank-mixture for all nozzles. From the highest to the lowest $Dv_{0.5}$ values followed pattern: dicamba + DRA 1> dicamba + DRA 2> dicamba. Similar observation as one defined for 138 and 207 kPa can be identified for TTI and ULD nozzle where the application of dicamba without DRAs resulted in slight droplet size value increase if compared with water-alone treatment. In general, it seems across evaluated treatments

OTM potential decrease of driftable fines and increase in $Dv_{0.5}$ values is constantly determined for dicamba with the addition of DRA 1. Further, at 276 kPa operating pressure the addition of DRA 2 into tank-mixture with TDXL-D nozzle type appears slightly to increase percentage of driftable fines compared with dicamba treatment having reduced efficacy based on their common use. On contrary, for TTI and ULD impact of the addition of DRAs can be observed. More particularly for TTI there was no difference identified among two DRAs tested, whereas for ULD nozzle type gradual decrease in percentage of driftable fines was observed.

Spray pattern study. Analysis of response variables across evaluated species revealed patterns distinctive per species evaluated as can be seen in Table 3. A significant solution*pressure interaction was observed influencing velvetleaf control between ($P<0.0001$) and underneath nozzles ($P=0.0039$). Whereas for common lambsquarters a solution*nozzle interaction was observed influencing control of the plants underneath the nozzles ($P<0.0441$) as well as operating pressure ($P<0.0125$). Common lambsquarters plants positioned between nozzles were influenced by solution, pressure, and nozzle factors significant on $\alpha=0.05$ level.

Velvetleaf. No differences in control were observed for velvetleaf plants positioned between nozzles for dicamba alone applications with 136, 207, and 276 kPa (Table 4). Across solution tested for 138 kPa the addition of DRA 1 in tank-mixture resulted in greater biomass reduction comparing with solution which contained DRA 2. However, with a pressure increase it appears dicamba tank-mixed with DRA 2 outperforms DRA 1 suggesting an inverse relationship among two DRAs tested as pressure increase. Further, combination of dicamba and DRA 2 greater biomass reduction for plants positioned between nozzles was detected with no difference among 207 and 276 kPa. Mean comparisons within each evaluated pressure shows similar pattern with DRA 2 as not as effective when using lower operated pressures while as pressure increased DRA 2 outperformed other two solution tested.

Across solution and operating pressures tested for velvetleaf plants positioned underneath the nozzles for both dicamba tank-mixed with DRA 1 or DRA 2 there is no difference was observed across 138, 207, and 276 kPa (Table 5). A mean comparison within each operating pressure shows dicamba plus DRA 2 outperformed other two tank-mixtures for 138 and 207 kPa. At the same time, mixture of dicamba plus DRA 1 for 138 kPa resulted in the lowest biomass reduction for plants positioned underneath nozzle. For 207 kPa the greatest biomass reduction was observed for dicamba treatment with DRA 2, with no difference among dicamba alone and dicamba with the addition of DRA 1. As pressure increased to 276 kPa treatment which contained DRA 1 was determined with a slightly lower dry biomass reduction compared with dicamba and dicamba with DRA 2.

Common Lambsquarters. Based on two-way significant (solution*nozzle) interaction following findings for biomass reduction are discussed combining data across pressures for plants positioned underneath nozzles (Table 6.) Application of treatments which included dicamba alone and dicamba with DRA 2 resulted in decrease of biomass reduction for TTI while no difference was observed among TDXL-D and ULD nozzle. However, it seems for dicamba with the addition of DRA 1 there is no difference in biomass reduction across nozzle type used. Treatment mean comparison within each nozzle type shows for TTI nozzle there is no difference observed among DRAs while at the same time the addition of DRAs in tank-mixture outperformed dicamba alone treatment. Findings associated with TDXL-D and ULD nozzle for dicamba tank-mixture which included DRA 2 always resulted in greatest biomass reduction. When dicamba alone treatment was applied over the top of common lambsquarters using a TDXL-D nozzle biomass reduction was 80%, whereas with the addition of DRA 1 and DRA 2 biomass reduction increased to 83 and 87%, respectively. Similar findings can be found with ULD nozzle type where the addition of DRA 2 with dicamba in tank-mix outperformed other tested combinations. Combining data across solution and pressures for plants positioned between nozzles (Table 7) as can be seen there is no difference in biomass reduction for applications with the TDXL-D and ULD nozzles whereas lower is expected when using TTI nozzle for application.

Impact of various pressures used difference for common lambsquarters plants positioned between and underneath nozzles can be seen in Table 8. For plants positioned between nozzles there was no difference among 207 and 276 kPa but lower biomass reduction in common lambsquarters plants is observed when applications are performed with low operated pressure as 138 kPa. Across pressures biomass reduction for plants underneath nozzles was determined to be different just among 138 and 207 kPa, having 82 and 83% of biomass reduction, respectively.

Solution affected biomass reduction for plants positioned between nozzles (Table 9). As can be seen result was dependent on DRA used in tank-mixture with dicamba. When dicamba alone was applied reduction of biomass was 80%; however, with the addition of either DRA 1 or DRA 2 plant biomass reductions increased to 84 and 87%, respectively.

Dose response study. As can be seen from Figure 2 common lambsquarters plant response was affected by tank-mixture used. Overall trend was as dicamba dose exponentially increased percentage of common lambsquarters biomass reduction increased. The parameters estimate for the log logistic biomass reduction are presented in Table 10. The lowest observed biomass reduction was associated when dicamba alone treatment was applied. The addition of DRAs increased biomass reduction having response distinctive among DRAs tank-mixed with dicamba. Results from this study suggests that DRA 2 is more effective for common lambsquarters biomass reduction. It is a noteworthy to mention that the addition of DRAs increased biomass reduction on common lambsquarters even when sublethal doses used. Based on parameter estimate ED_{50} (*e*) the effective dose to reduce 50% of plant biomass was 565.5, 114.3 and 81.2 g ae ha^{-1} for dicamba alone, dicamba plus DRA 1, and dicamba plus DRA 2, respectively.

Discussion

Primary OTM mitigation practices include modifications of DSD through the employment of different tactics which as a result have an increase in droplet size. Overall findings from this study suggests nozzle type, operated pressure, and DRAs used in tank-mixture with dicamba can considerably impact DSD and OTM potential. As reported in literature the greatest impact on generated droplets can be expected from nozzle types and operated pressure used rather than spray mixture (Dorr et al. 2013). As seen across 138, 207, and 276 kPa pressures, $Dv_{0.5}$ values for evaluated drift-reducing nozzles followed the pattern: TTI>TDXL-D>ULD from highest to lowest, respectively. Further, as operated pressure increased DSD parameters ($Dv_{0.1}$, $Dv_{0.5}$, $Dv_{0.9}$) decreased with greater quantity of driftable fines (<200 µm). Both inverse and positive association among pressure with droplet diameter and driftable fines have been reported in literature, respectively (Nuyttens et al. 2007).

It is reported changes in DSD or spray classification can occur even with the addition of either herbicide or various agents in tank-mixture (Creech et al. 2015, Oliveira et al. 2015). Even though, findings in present study shows UC spray classification for all evaluated treatments the addition of DRAs in tank-mixture with dicamba increased $Dv_{0.5}$ and decreased of driftable fines considerably with final response dependent on nozzle used. Majority of evaluated treatment combinations resulted as effective for OTM mitigation when DRAs was added into tank-mixture with dicamba. However, slight increase in driftable fines was observed with dicamba application at 276 kPa when DRA 2 was in tank-mixture with dicamba for TDXL-D nozzle likely as result of the different design structure of this nozzle. Johnson et al. (2006) found drift-reducing nozzles sometimes can be more effective approach than including DRAs in tank-mixture under certain scenarios. Based on findings in present study the use of drift-reducing nozzles is necessary to prevent OTM of dicamba; however, as shown the most effective approach for OTM mitigation was observed when DRAs were present with dicamba in tank-mixture for majority of evaluated treatments.

Different nozzle types and their impacts on pesticide application and formation of spray liquid sheet can be attributed partially with their design. The modification of the solution physical properties can affect the atomization process, spray formation, and treatment performance (Dorr et al. 2013, Hewitt 2008). Application of solution with altered physical characteristics when DRA 1 and DRA 2 were added with dicamba for TDXL-D and ULD nozzle types especially with application at 138 kPa may not result in proper fan formation and cause nozzle collapses (Figure S1.1 – Figure S1.3). It seems more likely that the TDXL and ULD nozzle should not be used at lower pressure with thickening type DRAs as they do not perform as they should. Similar findings have been reported where lower operational pressures resulted in spray pattern variability or decrease in nozzle exiting fan-angle (Dorr et al. 2013, Etheridge et al. 1999). Decreased fan-angles may directly influence proper overlapping and deliver lower dose of herbicide than anticipated on targeted weed species. Considering that fan-angle was not developed fully it fill be necessary for future studies to evaluate additional metrics that will help with identification if the lack of the spray development occurs just at 138 kPa or it can affect as well operating pressures 207 and 276 kPa.

Previous research demonstrated greater efficacy of some products can be explained with an inverse association among droplet size, efficacy, and deposition as droplet size decreases deposition and herbicide efficacy increase (Knoche 1994, Derksen et al. 1999). However, with dicamba this is not feasible due to OTM concerns. Although increase in droplet size decreases OTM potential a reduction in terms of weed control may be expected (Knoche 1994, Wolf 2002). Even though increase in droplet size may be a primary factor that reduce drift potential various herbicides may have different performance. For example, glyphosate - other systemic products with a high-potential for various OTM droplet size was not observed to have an impact on weed control across several evaluated species when droplet size was increased (Feng et al. 2003, Ferguson et al. 2018). This research does emphasize that glyphosate absorption, translocation, and efficacy was observed even with coarse or ultra-coarse spray droplet sizes.

In general, the performance of any applied pesticide has a direct relation with the quantity of products deposited on the leaves of targeted plant. The addition of DRAs in tank-mixture resulted in droplet size increase. For TTI nozzle type at 138 kPa $Dv_{0.5}$ value increased from 1078 to 1193 µm comparing dicamba and dicamba + DRA 1, respectively. Increase in droplet size like this can be critical component for biological activity of herbicides where for every 100 µm droplet size increase it can be expected decrease in herbicide deposition (Smith et al. 2000). Further, the presence of various natural leaf obstacles can impact spray deposition and performance of applied herbicides (Sanyal et al. 2006). Reduced droplet retention on the leaf surface can be directly associated with a fate of the droplets during and after herbicide application process occur. After droplets reach surface, they will either retain, bounce or roll off the leaf surface if there is any sort of incompatibility of sprayed solution with targeted surface (Aytouna et al. 2010, Hess et al. 1974).

The distinct leaf surface characteristics between velvetleaf (i.e. trichomes) and common lambsquarters (i.e. epicuticular wax) can affect pesticide retention and further uptake. Adjuvants including surface-active agent, wetting agent, foliar retention, and/or deposition aid agents are often used with herbicides to overcome those leaf barriers and therefore increase the application performance on-target plants (Aytouna et al. 2010, Riechers et al. 1994, Sanyal et al. 2006). Even though, the DRAs tested in this study are primarily used for drift mitigation, their purpose is often cross listed with other functions indirectly facilitating biological activity of pesticides (Anonymous 2017, Anonymous 2018)

Velvetleaf plants positioned either below or underneath the nozzle was very sensitive to dicamba application of 560 g ae ha^{-1} based on biomass reduction findings. Biomass reduction observed for velvetleaf was reported not lower than 94%. All changes in DSD caused with the addition of DRAs in tank-mixture had a minor impact on overall treatment performance. Similar findings were reported in literature where applications of dicamba on velvetleaf were not influenced by spray classification changes from fine to extremely coarse (Creech et al. 2016). One of the concerns with POST herbicide activity with species like velvetleaf is droplet contact angle with leaf surface due to presence of trichomes as natural obstacle (Hess et al. 1974). According to (Sanyal et al. 2006) issues with spread area were overcome with the addition of non-ionic surfactant at rate of 0.25% v v^{-1} which in present study was accomplished by adding multipurpose DRAs in tank-mixture.

Based on this study findings the presence of leaf natural barrier on common lambsquarters resulted in decrease of biomass reduction. It is well known the presence of hard to wet surface can impact retention of majority of POST herbicides used in row crops. Several studies showed an inverse relationship among amount of epicuticular wax on leaf surface with contact angle of droplets and herbicide efficacy (Ramsdale and Messersmith 2001, Sanyal et al. 2006). For herbicides like dicamba efficacy can be influenced by droplet size (Butts et al. 2018). Also, Creech et al. (2016) findings shows dicamba efficacy on common lambsquarters can be reduced efficacy when droplet size increased from fine (F) to extremely course (XC) spray classification. Additionally, based on dose response study findings the addition of DRAs in dicamba tank-mixture was necessary to improved common lambsquarters control with distinct response among adjuvants used. As seen for common lambsquarters control with respect to solution used followed the pattern: dicamba + DRA 2 > dicamba + DRA 1> dicamba from highest to lowest biomass reduction, respectively.

Interaction among herbicide used and targeted weed species needs to be considered as a complex process which is often site-specific. In order to maximize performance when dicamba or other systemic products needs to be evaluated across multiple weed species (Butts et al. 2019, Creech et al. 2016, Feng et al. 2003). Considering complexity of common lambsquarters response associated with dicamba performance when various formulation of DRAs were used requires additional research.

Conclusions

The results observed in this research shows nozzle selection, solution, and operating pressure needs to be considered as critical component for both DSD and herbicide efficacy. Consistent performance of TTI nozzle type in terms of DSD was determined regardless scenario tested with the largest droplet size values with lowest driftable fines generated. Even though, OTM using a TTI nozzle type was reduced a negative impact on biological efficacy was observed. In general, the greatest biomass reduction was observed for nozzles which DSD included smaller $Dv_{0.5}$ and $Dv_{0.9}$ followed with greater $DV_{0.1}$ and driftable fines values. Applications with low operating pressures for nozzle types TDXL-D and ULD are not recommended since with thickening type DRAs as they do not perform as they should resulting in lack of proper fan formation having a possibility for plant biomass reduction positioned between and underneath nozzles to be affected. Despite the spray pattern issues the addition of DRAs in tank-mixture with dicamba improved weed control. The lack of proper fan development at low operational pressures was overcome with pressure increase. Velvetleaf plants regardless position had a biomass reduction over 94%. On contrary, hard to wet surface like one on common lambsquarters had biomass reduction ranging from 74 to 87%. Variation among species tested in this study could be explained by their morphological characteristics, although further research on this topic is needed. Mitigation of OTM issues with dicamba to decrease environmental contamination is required, however a better understanding about advantages and disadvantages when multiple mitigation practices are used represents a critical step to identify combination that will extend lifetime of chemistry use and maximize weed control.

List of Tables

Table 1. Analysis of variance for effects of dicamba solution, pressure, nozzle, and their interactions with each other on droplet size parameters.

	Droplet size parameter[a]				
Factor	$DV_{0.1}$	$DV_{0.5}$	$DV_{0.9}$	RS	Driftable fines
	---------- p-value[b] ----------				
Solution	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pressure	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Nozzle	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Solution x Pressure	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Solution x Nozzle	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pressure x Nozzle	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Solution x Pressure x Nozzle	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] Definitions: $DV_{0.1}$, $DV_{0.5}$, and $DV_{0.9}$, parameters that represent the droplet size such that 10, 50, and 90% of the spray volume is contained in droplets equal or lesser values, respectively; driftable fines, percent of spray volume that contains driftable fines <200 µm; RS, relative span, a dimensionless parameter that estimates the spread of a distribution.

[b] Significant at $\alpha = 0.05$

Table 2.1. Droplet size distribution of four solutions sprayed through three nozzle types at 138 kPa pressure.

		Droplet size parameter[a]				
Nozzle	Solution	$DV_{0.1}$	$DV_{0.5}$	$DV_{0.9}$	RS	Driftable fines
		----------------------------	µm	-------------------------		%
TTI 11004	Water	539 G	983 J	1353 F	0.83 D	0.24 CB
	Dicamba	588 D	1078 F	1515 D	0.86 B	0.19 CDE
	Dicamba + DRA 1	681 A	1193 B	1625 C	0.79 FE	0.11 F
	Dicamba + DRA 2	656 B	1178 C	1630 C	0.83 D	0.14 FE
TDXL 11004-D	Water	582 D	1028 H	1384 E	0.78 F	0.18 DE
	Dicamba	564 F	998 I	1359 F	0.80 E	0.20 CD
	Dicamba + DRA 1	674 A	1215 A	1718 A	0.86 B	0.05 G
	Dicamba + DRA 2	604 C	1102 E	1531 D	0.84 C	0.15 FDE
ULD 12004	Water	486 I	880 L	1216 H	0.83 D	0.40 A
	Dicamba	509 H	905 K	1267 G	0.84 DC	0.38 A
	Dicamba + DRA 1	574 E	1043 G	1403 E	0.79 E	0.20 CD
	Dicamba + DRA 2	603 C	1155 D	1661 B	0.92 A	0.26 B

Means within a column followed by the same letter are not significantly different ($P \leq 0.05$).

[a] Definitions: $DV_{0.1}$, $DV_{0.5}$, and $DV_{0.9}$, parameters that represent the droplet size such that 10, 50, and 90% of the spray volume is contained of droplets of lesser diameter, respectively; driftable fines, percent of spray volume that contains driftable fines <200 µm; RS, relative span, a dimensionless parameter that estimates the spread of a distribution.

Table 2.2. Droplet size distribution of four solutions sprayed through three nozzle types at 207 kPa pressure.

		Droplet size characteristics[a]									
Nozzle	Solution	$DV_{0.1}$		$DV_{0.5}$		$DV_{0.9}$		RS		Driftable fines	
		----------------------------		µm		-------------------------				%	
TTI 11004	Water	466	G	891	F	1305	FE	0.94	A	0.60	B
	Dicamba	504	E	954	D	1345	D	0.88	C	0.42	D
	Dicamba + DRA 1	620	A	1113	A	1552	A	0.84	E	0.20	F
	Dicamba + DRA 2	593	B	1076	B	1468	B	0.81	F	0.23	F
TDXL 11004-D	Water	507	ED	911	E	1286	F	0.86	D	0.37	ED
	Dicamba	470	G	861	G	1201	G	0.85	ED	0.64	B
	Dicamba + DRA 1	538	C	990	C	1365	C	0.84	E	0.35	E
	Dicamba + DRA 2	511	D	965	D	1355	DC	0.88	C	0.40	ED
ULD 12004	Water	411	I	752	I	1091	H	0.91	B	0.94	A
	Dicamba	424	H	774	H	1103	H	0.88	C	0.97	A
	Dicamba + DRA 1	493	F	914	E	1296	F	0.88	C	0.48	C
	Dicamba + DRA 2	470	G	908	E	1316	E	0.93	A	0.52	C

Means within a column and nozzle followed by the same letter are not significantly different ($P \leq 0.05$).

[a] Definitions: $DV_{0.1}$, $DV_{0.5}$, and $DV_{0.9}$, parameters that represent the droplet size such that 10, 50, and 90% of the spray volume is contained of droplets of lesser diameter, respectively; driftable fines, percent of spray volume that contains driftable fines <200 µm; RS, relative span, a dimensionless parameter that estimates the spread of a distribution.

Table 2.3. Droplet size distribution of four solutions sprayed through three nozzle types at 276 kPa pressure.

Nozzle	Solution	Droplet size characteristics[a]				
		$DV_{0.1}$	$DV_{0.5}$	$DV_{0.9}$	RS	Driftable fines
		---------------------------- µm	-------------------------			%
TTI 11004	Water	399 G	797 G	1184 E	0.98 A	1.14 C
	Dicamba	441 D	869 D	1285 C	0.97 A	0.80 G
	Dicamba + DRA 1	547 A	1010 A	1403 A	0.85 F	0.37 H
	Dicamba + DRA 2	537 B	994 B	1379 B	0.85 F	0.39 H
TDXL 11004-D	Water	439 D	814 F	1151 F	0.88 E	0.81 G
	Dicamba	421 F	784 H	1115 G	0.89 D	0.98 E
	Dicamba + DRA 1	457 C	890 C	1292 C	0.94 B	0.91 F
	Dicamba + DRA 2	424 F	832 E	1208 D	0.94 B	1.05 D
ULD 12004	Water	367 I	686 J	1008 I	0.94 B	1.46 A
	Dicamba	387 H	729 I	1072 H	0.94 B	1.28 B
	Dicamba + DRA 1	432 E	839 E	1184 E	0.90 D	1.12 C
	Dicamba + DRA 2	425 F	836 E	1198 ED	0.92 C	1.02 ED

Means within a column and nozzle followed by the same letter are not significantly different ($P \leq 0.05$).

[a] Definitions: $DV_{0.1}$, $DV_{0.5}$, and $DV_{0.9}$, parameters that represent the droplet size such that 10, 50, and 90% of the spray volume is contained of droplets of lesser diameter, respectively; driftable fines, percent of spray volume that contains driftable fines <200 µm; RS, relative span, a dimensionless parameter that estimates the spread of a distribution.

Table 3. Analysis of variance for effects of dicamba solution, pressure, nozzle, and their interactions with each other on percentage of biomass reduction of two weed species.

Factor	Velvetleaf		Common lambsquarters	
	Between nozzles	Underneath nozzles	Between nozzles	Underneath nozzles
	-- p-value[a]	--		
Solution	0.4083	0.0010	<0.0001	<0.0001
Pressure	0.6490	0.1001	<0.0001	0.0125
Nozzle	0.7912	0.6121	<0.0001	<0.0001
Solution x pressure	<0.0001	0.0039	0.9378	0.8897
Solution x nozzle	0.7895	0.8375	0.1886	0.0441
Pressure x nozzle	0.6806	0.9908	0.7106	0.9491
Solution x pressure x nozzle	0.8886	0.9970	0.9420	0.9967
Coefficient of variation (%)	4.25	4.29	6.59	7.36

[a] Significant at $\alpha = 0.05$

Table 4. Biomass reduction of velvetleaf positioned between nozzles after being exposed to different dicamba solutions sprayed at three pressures. Data combined across nozzles.

Solution	Pressure (kPa)		
	138	207	276
	---------------------------------------	%	---------------------------------------
Dicamba	95.4 aA	94.8 aB	95.3 aA
Dicamba + DRA 1	95.5 aA	95.1 abB	94.6 bB
Dicamba + DRA 2	94.6 bB	96.1 aA	95.7 aA

Means followed by the same letter, lower case in the row and upper case in the column, do not differ using Tukey's test at α = 0.05.

Table 5. Biomass reduction of velvetleaf positioned underneath nozzles after being exposed to different dicamba solutions sprayed at three pressures. Data combined across nozzles.

Solution	Pressure (kPa)		
	138	207	276
	-------------------------------------	%	-------------------------------------
Dicamba	95.1 aB	94.3 bB	95.0 aA
Dicamba + DRA 1	94.5 aC	94.6 aB	93.9 aB
Dicamba + DRA 2	95.7 aA	96.0 aA	95.4 aA

Means followed by the same letters, lower case in the row and upper case in the column, do not differ using Tukey's test at α = 0.05.

Table 6. Biomass reduction of c. lambsquarters positioned underneath nozzles after being exposed to different dicamba solutions sprayed through three nozzle types. Data combined across pressures.

Solution	Nozzle		
	TTI	TDXL-D	ULD
	--	---%---	--
Dicamba	73.6 bB	80.4 aC	80.6 aC
Dicamba + DRA 1	81.8 aA	83.2 aB	83.3 aB
Dicamba + DRA 2	83.9 bA	87.7 aA	88.7 aA

Means followed by the same letter, lower case in the row and upper case in the column, do not differ using Tukey's test at α = 0.05.

Table 7. Biomass reduction of common lambsquarters positioned between nozzles treated with dicamba sprayed through three nozzle types. Data combined across solutions and pressures.

Nozzle	Biomass reduction	
	---------------------------------%---------------------------------	
TTI	81.3	B
TDXL	84.4	A
ULD	85.1	A

Means followed by the same letter in the column do not differ using Tukey's test at α = 0.05.

Table 8. Biomass reduction of c. lambsquarters positioned between and underneath nozzles treated with dicamba sprayed at three pressures. Data combined across solutions and nozzles.

Pressure	Between Nozzle		Underneath Nozzle	
kPa	---%		--	
138	82.0	B	81.7	B
207	84.6	A	83.4	A
276	84.2	A	82.8	AB

Means followed by the same letter in the column do not differ using Tukey's test at α = 0.05.

Table 9. Biomass reduction of c. lambsquarters positioned between nozzles treated with dicamba solutions. Data combined across pressures and nozzles.

Solution	Biomass reduction
	--------------%---------------
Dicamba	79.7 C
Dicamba + DRA 1	83.7 B
Dicamba + DRA 2	87.3 A

Means followed by the same letter in the column do not differ using Tukey's test at α = 0.05.

Table 10. Log-logistic model parameter estimates and SEs for common lambsquarters biomass reduction (%) regressed over dicamba doses (g ae ha^{-1}) for dicamba applications without and with drift-reducing agent (DRA).[a]

Solution	*b* (SE)	*c* (SE)	*d* (SE)	*e* (SE)
Dicamba	-2.39 (0.64)	16.55 (1.14)	78.06 (10.42)	565.49 (112.56)
Dicamba + DRA 1	-2.21 (0.60)	21.14 (1.41)	75.61 (3.32)	114.34 (16.05)
Dicamba + DRA 2	-1.24 (0.21)	19.32 (2.14)	86.73 (3.51)	81.19 (11.32)

[a] The *b* is the slope at the inflection point, *c* is the lower limit of model, *d* is the upper limit, and *e* is the inflection point (GR_{50} – the effective dose to reduce 50% of plant biomass).

Supplemental List of Tables

Table S1. Normality of residuals and homogeneity of variance of biomass reduction data based on plant position in relation to the nozzle.

Species	Plant position	Test	Original data		Transformed data			Analysis[b]
			F/KS values[a]	Significance	Type of transformation	*F/KS values*	Significance	
Velvetleaf	Between	Levene	3.073	<0.0001	asin(√x/100)	2.963	<0.0001	NT
		KS	0.091	<0.0001		0.083	<0.0001	
	Underneath	Levene	3.226	<0.0001	asin(√x/100)	3.311	<0.0001	NT
		KS	0.083	<0.0001		0.050	0.046	
Common lambsquarters	Between	Levene	1.682	0.022	-			NT
		KS	0.038	0.200				
	Underneath	Levene	1.312	0.146	-			NT
		KS	0.027	0.200				

[a] F and K-S values of the F statistic for the Levene's test and K-S for the Kolmogorov-Smirnov's test, respectively.

[b] NT: non-transformed

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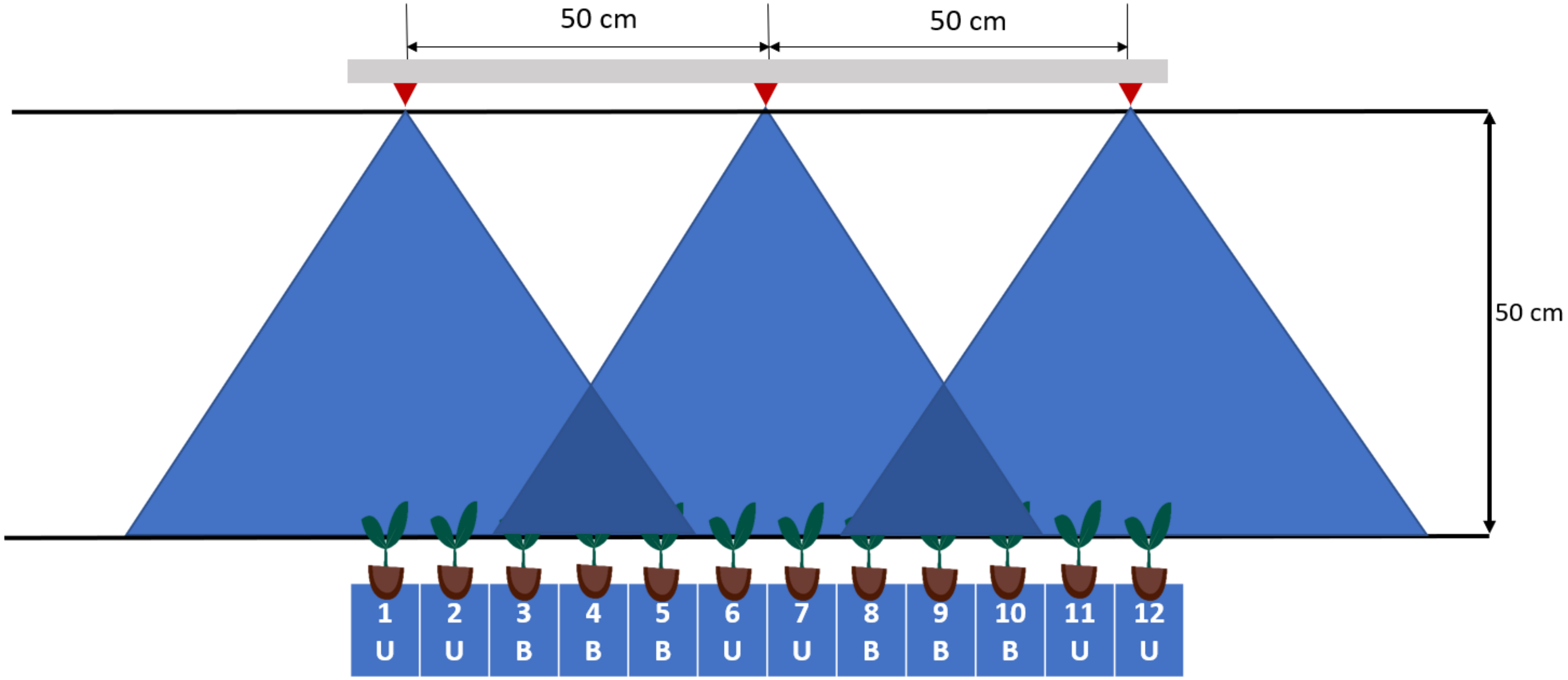

Figure 1. Three-nozzle spray chamber layout showing the position of plants, underneath (U) and between (B) nozzles, during dicamba applications.

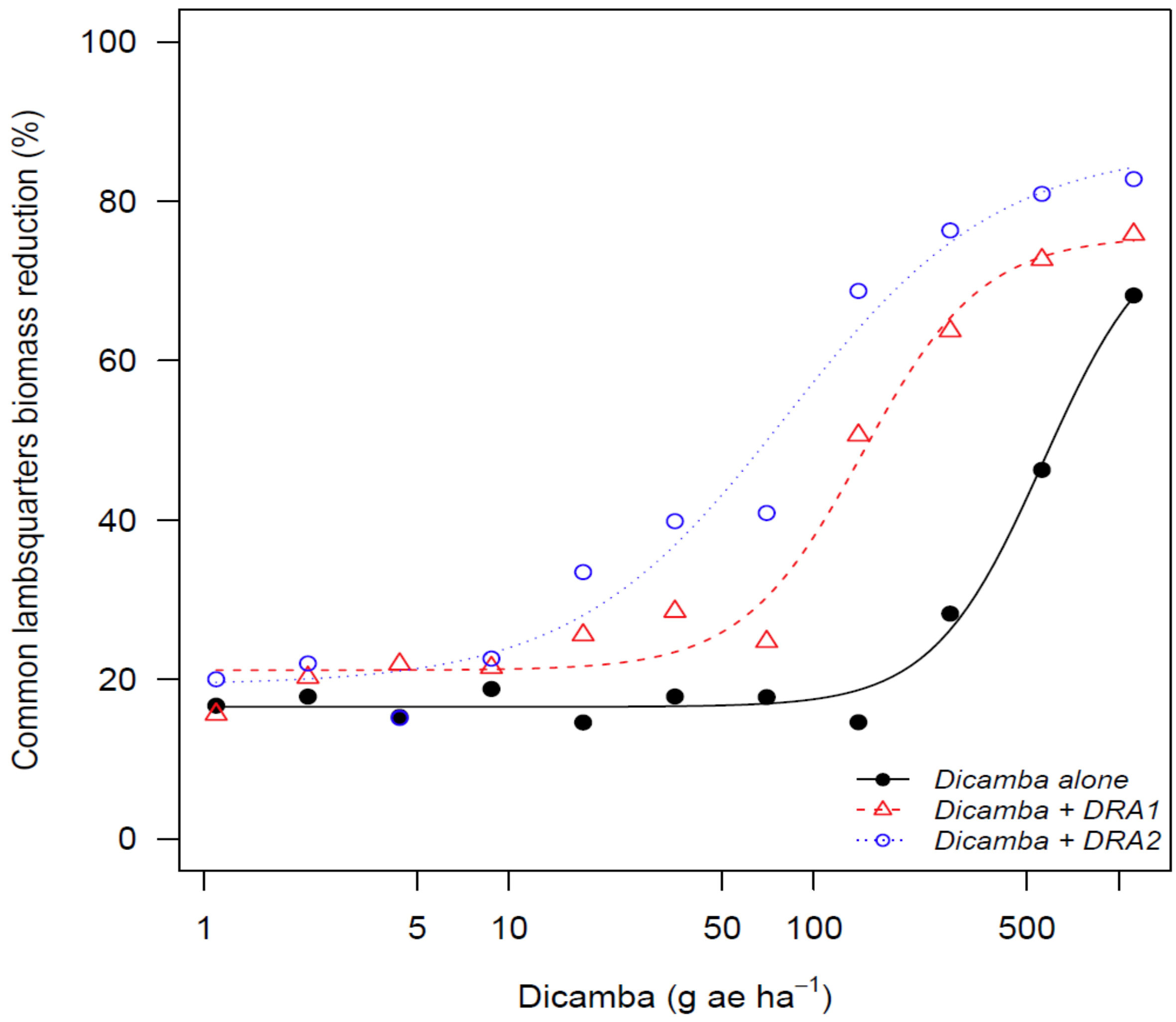

Figure 2. Common lambsquarters biomass reduction (%) as influenced by dicamba solutions without or with the addition of drift reducing agents (DRAs).

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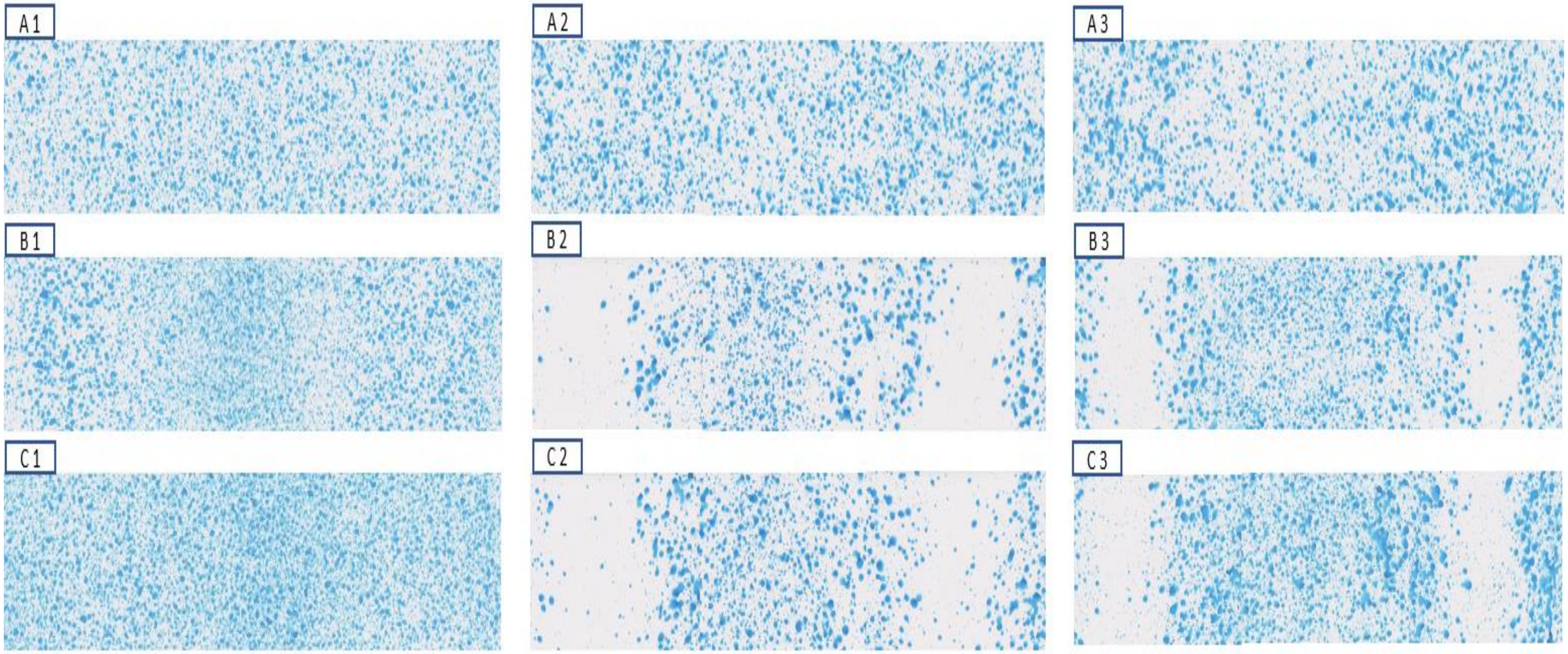

Figure S1.1. Spray pattern evaluation for TTI (A), TDXL-D (B), ULD (C) with dicamba alone (1), dicamba and DRA 1 (2), dicamba and DRA 2 (3) at 138 kPa.

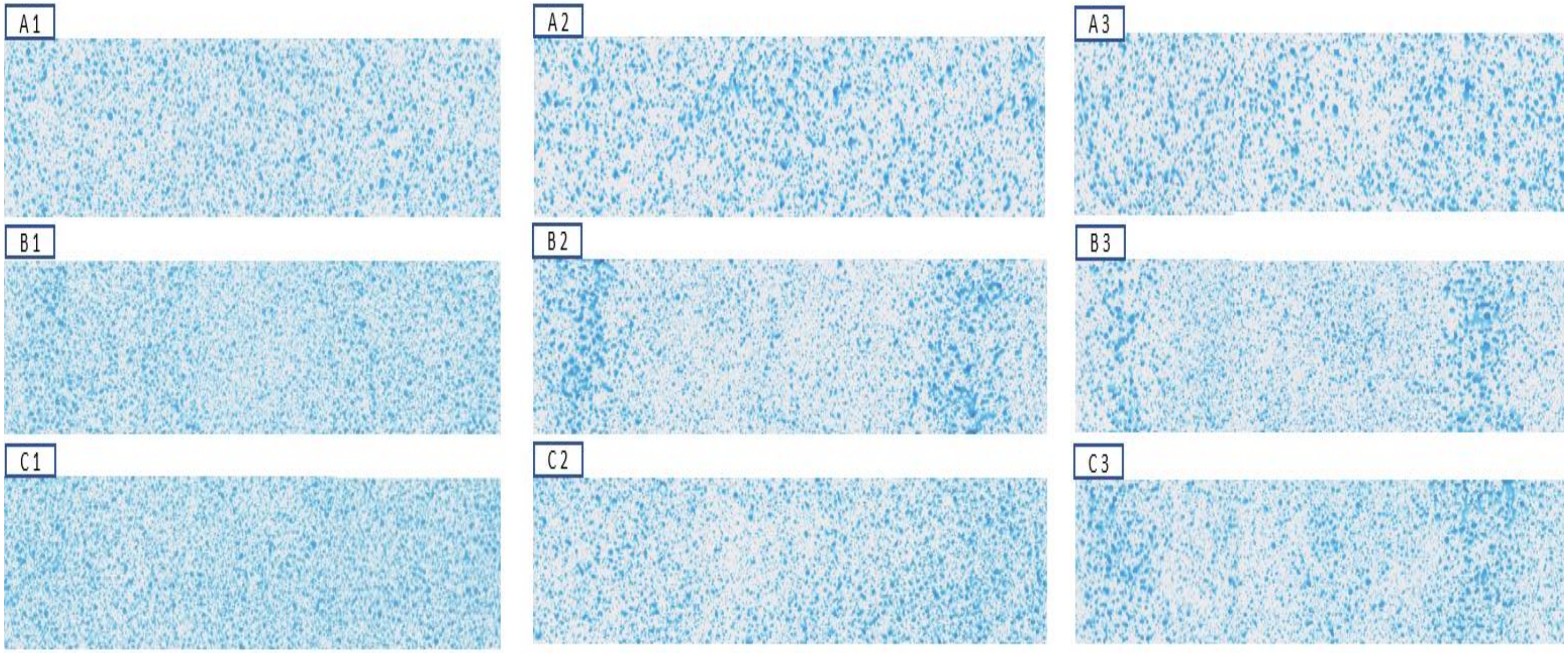

Figure S1.2. Spray pattern evaluation for TTI (A), TDXL-D (B), ULD (C) with dicamba alone (1), dicamba and DRA 1 (2), dicamba and DRA 2 (3) at 207 kPa.

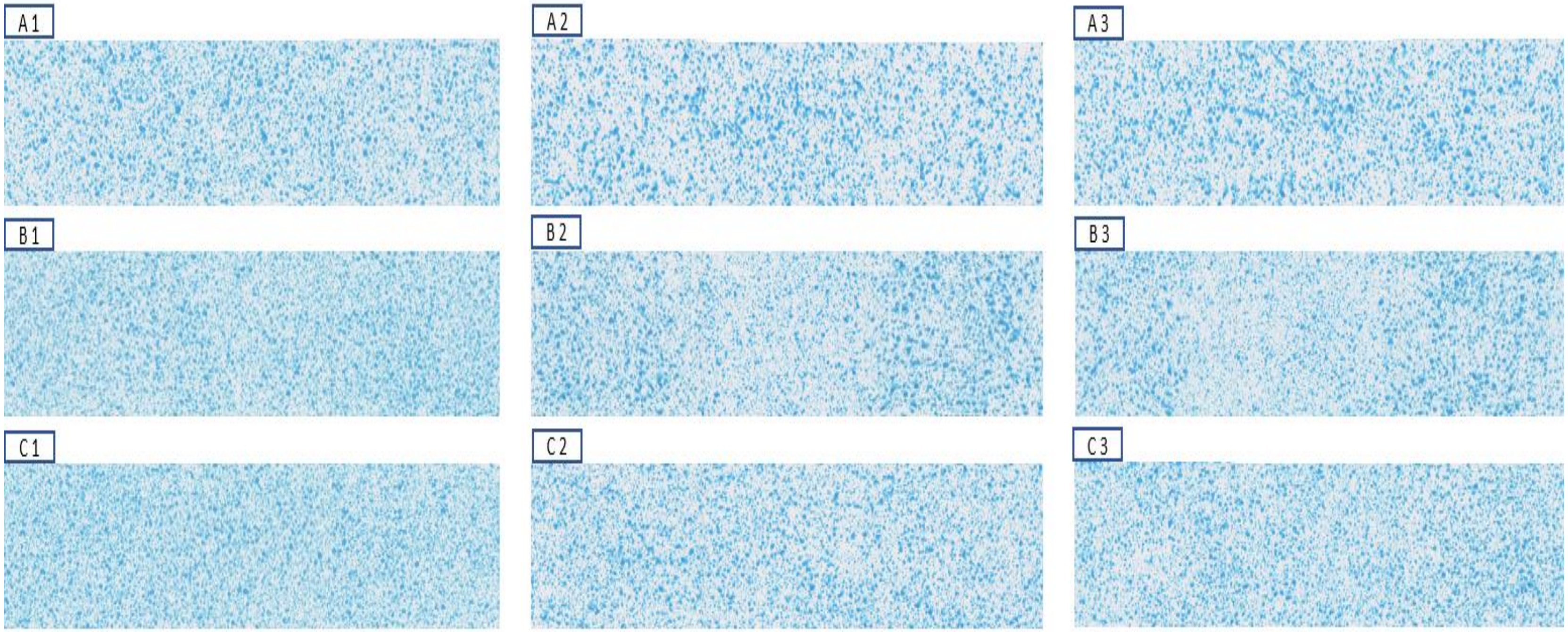

Figure S1.3. Spray pattern evaluation for TTI (A), TDXL-D (B), ULD (C) with dicamba alone (1), dicamba and DRA 1 (2), dicamba and DRA 2 (3) at 276 kPa.

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